ANALYTICAL AND STRUCTURAL STUDIES OF PLANT GUM EXUDATES

By

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TO: LiangLiang and those
who gave their great help and kindness
during 1988-1992
and
my Motherland

Some of the analytical data reported in this Thesis have been published, eg.

- D.M.W.Anderson and Wang Weiping, **Food Hydrocolloids**, 1990, 3, 475-484."The characterization of *Acacia paolii* gum and four commercial *Acacia* gums from Kenya".

- D.M.W.Anderson, Wang Weiping and G.P.Lewis, **Biochemical Systematics and Ecology**, 1990, 18, 39-42."The composition and Properties of Eight Gum Exudates(Leguminosae) of American Origin".

- D.M.W.Anderson and Wang Weiping, **Biochemical Systematics and Ecology**, 1990, 18, 43-44. "The composition of some *Sesbania* Gum Exudates".

- D.M.W.Anderson and Wang Weiping, **Phytochemistry**, 1990, 29, 1193-1195."The composition of the gum from *Combretum paniculatum* and other gums which are not permitted food additives".

- D.M.W.Anderson and Wang Weiping, **Biochemical Systematics and Ecology**, 1990, 18, 413-418."*Acacia* gum exudates from Somalia and Tanzania; the *Acacia senegal* complex".

- D.M.W.Anderson and Wang Weiping, **Food Hydrocolloids**, 1991, 5, 297-306. "The characterization of gum arabic samples from Uganda".

- D.M.W.Anderson, J.R.A.Millar and Wang Weiping, **Biochemical Systematics and Ecology**, 1991, 19, 447-452."Gum Arabic from Niger --Comparison with other Sources and Potential Agroforestry Development".

- D.M.W.Anderson, A.Stefani and Wang Weiping, **International Tree Crops Journal**, 1991, 6, 275-285."*Combretum nigricans* Gum --- Its unusual structure/properties and differences from gum arabic".

- D.M.W.Anderson, J.R.A.Millar and Wang Weiping, **Food Additives and Contaminants**, 1991, 8, 423-436."The gum exudate from *Combretum nigricans* gum, the major source of West African 'gum combretum'".

- D.M.W.Anderson and Wang Weiping, **International Tree Crops Journal**, 1991, 7, 29-40."*Acacia seyal* and *Acacia sieberana* --sources of commercial gum talha in Niger and Uganda".

Some studies to which I contributed during this period of study, but are not included in this Ph.D. Thesis, have been published and are presented in reprint form eg.

- D.M.W.Anderson, D.M.Brown Douglas, N.A.Morrison and Wang Weiping, **Food Additives and Contaminants**, 1990, 7, 303-321. "Specifications for gum arabic; analytical data for samples collected between 1904 and 1989".

- D.M.W.Anderson, J.R.A.Millar and Wang Weiping, **Food Additives and Contaminants**, 1991, 8, 405-421."Gum Arabic: unambiguous identification by ^{13}C-NMR spectroscopy as an adjunct to the Revised JECFA Specification and the application of ^{13}C-NMR spectra for regulatory/legislative purposes".

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ABSTRACT

Studies presented in this Thesis fall into four distinct groups:

1. Analytical characterizations have been made of the gum exudates from: (a) Eight species of the Series Gummiferae and Vulgares and three highly proteinaceous species of the Series Juliflorae of the genus *Acacia*; (b) Six species from the genus *Leucaena* which are chemically very close to gum arabic; (c) Nine specimens of gum obtained from *Combretum nigricans* growing in different locations; (d) Six *A. senegal* gum samples from Uganda and a further six "gum arabic" samples from different regions; (e) Seventeen species of gum exudates from 15 different genera such as *Cassia grandis, Cercidium praecox, Parkia nitida, Prosopis chilensis, Sesbania sesban, Atalaya hemiglauca, Sclerocarya birrea, Pseudocedrela kotschyi, Senna nicaraguensis,* etc..

2. A study of some of the structural features of *Combretum nigricans* gum by Smith-degradation showed that uronic acid and rhamnose groups occur in internal locations within the overall structure, and not as end-groups as is the case in gum arabic (*A. senegal*).

3. Amino acid data and ^{13}C NMR spectra are presented for various fractions of *A. senegal* gum and for some highly proteinaceous *Acacia* gums (e.g. *A. difficilis, A. eriopoda, A. tumida*). The effect of enzymes on these gums is reported.

4. ^{13}C NMR spectra for thirty different gum exudates are presented to show the characteristic "fingerprint" patterns given by their polysaccharide structures. Information concerning their component monosaccharides, anomeric configurations, and linkages between the various sugar residues is given by spectrum analysis. This provides a most sensitive way to identify botanical species based on the total structure of exuded gums. The arabinose form (pyranose or furanose) and the various linkage configurations (α or β) in the gum structures are important and directly affect the physico-chemical behaviour of gum exudates. *Acacia* gums in which rhamnose is absent (or nearly absent) generally have a large proportion of β-L-arabinopyranose (*A. seyal, A. sieberana, A. arabica* gums etc.). The major arabinose form is α-L-arabinofuranose in gum arabic (*A. senegal*) whereas it is β-L-arabinopyranose in gum tahla (*A. seyal*).

Contents

Chapter 1
General Introduction

Water-soluble gums of plant origin represent an enormous quantity of industrial material supplied by plant growing areas in most parts of the world (Whistler 1973). Gums come from a variety of botanical species. They are used not only in the food industry but also in the adhesive, paper, textile, cosmetic, pharmaceutical and other industries (Davidson 1980).

Gums are seldom used alone in applications but are almost always mixed with other materials. Their principal role is to control physical properties. They are excellent suspending agents, dispersants, stabilizing agents, emulsifiers and gel-forming agents. Structurally, they are mainly complex, highly branched carbohydrate polymers composed of sugar units glycosidically linked to form large molecules which are generally linked to small amounts of protein.

Many plant families exude gums in greater or lesser degree. Gums may either be exuded in only very small quantities from some plant species, or they may be produced very copiously, forming large, conspicuous incrustations. It was reported that exudation usually follows mechanical injury or bacterial infestation of the bark (Smith and Montgomery 1959), and bacterial action, enzymatic conversion of starch or hemicellulose, and direct synthesis, have all been mentioned as possible explanations (Whistler 1973); even the location of the site of gum formation is reported to be important (Joseleau and Ullmann 1990). But the precise mechanism of gum formation is still not fully understood. It is believed that a more reasonable explanation is that mechanical injury (or tapping) of gum trees only allows the gum to exude from the tree i.e the gum is an important physiological function of the tree allowing the tree to hold water to maintain its normal metabolism under water-depletion stress in arid areas. Functionally, plant gums may also act to seal off wounds inflicted by wood-boring insects, grazing animals etc. and thus form a protection against tissue dehydration. The term "gum" designates a variety of natural products in the form of flakes, tears or angular fragments, sticky in nature and deposited on the surface of the plants. Those plants that produce commercial exudate gums are usually shrubs or low-growing trees that can survive in arid desert areas. Harvesting is by hand picking. After collection in sufficient quantities, the gum is taken to a central collection area where it is hand-sorted into different grades, packaged and shipped.

The plant gums have previously been regarded as complex, acidic, hetero-polysaccharides exuded from the stems and branches of certain tropical and sub-tropical species. They are commonly found in the Sahelian zone of Africa, Australia, India, South America and parts of Asia. In the aqueous phase the gums produce viscous dispersions or gels. They are regarded as hydrocolloids as they rarely give true solutions. Gum arabic is one of the most important and widely utilized edible gums and originates, by definition, from *Acacia senegal* (L.) Willd. It is a highly water-soluble gum of relatively low viscosity. It is the only *Acacia* gum to have been toxicologically tested to establish its safety as a food additive. Its main foodstuff applications include confectionery, medicated lozenges/pastilles, encapsulation of dried flavours/fragrances, and the emulsification of citrus oils for use in soft drinks etc. (Anderson 1989). In addition, the poorer, darker grades of gum arabic are used in technological applications.

Chemically, the gums occur as mixed salts of partially neutralised complex polysaccharide acids containing pentose, hexose, methylpentose and uronic acid units linked together in a complex, highly branched manner. The neutral sugars most commonly found are β- and α-D-galactose, L-arabinofuranose and L-arabinopyranose (both α and β anomers) and α-L-rhamnose in *Acacia* species, but D-xylose, D-mannose and D-glucose are present in some species (not in *Acacia*). The acidity of the gums arises from the presence of varying quantities of D-glucuronic acid and its 4-O-methyl derivative, but some genera (not *Acacia*) also contain D-galacturonic acid (Anderson et al. 1972). Some exudate gums (e.g. gum arabic) are highly soluble in water, giving viscous solutions; others do not dissolve completely and give suspensions or gels.

The presence of peptides/proteins in gum exudates and the amino acid composition of gum arabic was first reported in 1972 (Anderson et al. 1972). A small proportion (usually under 5%) of proteinaceous material is always present in most gums, although some species e.g. *Acacia difficilis* gum contains up to as much as 56%. This proteinaceous material is possibly important structurally and rheologically (Akiyama 1984). Therefore it is more correct to refer to these plant gums as proteoglycans rather than as polysaccharides. Early attempts to isolate the proteinaceous material without causing extensive degradation to the polysaccharide were unsuccessful (Anderson and Hendrie 1971; Anderson et al. 1972), although Phillips and co-workers have reported that a fraction of gum arabic (1% yield) containing 50% protein has been obtained (Menzies et al. 1991).

In 1961, gum arabic was classified "GRAS" by the U.S. Food and Drug Administration as a food stabiliser (Anderson 1977; Anderson 1984) after a study of its safety in terms of the relevant scientific reports available. Gum arabic was re-affirmed in 1974 as Generally Regarded As Safe (GRAS) for direct use in foodstuffs; and the "not specified" category of Acceptable Daily Intake (ADI) was assigned by the EEC in 1982 after submissions of reports of extensive studies carried out in animals and in man at Edinburgh University (Anderson et al. 1982). In contrast, the gums from none of the other *Acacia* species (over 1100 have been identified) have ever been subjected to food safety trials. Strictly, they are therefore not permitted as food additives. However trade misdescriptions, both accidental and deliberate, are not uncommon (Anderson 1978a), and such gums have been used as adulterants for gum arabic by unscrupulous traders who appear to be unwilling to implement the required principles of good manufacturing practice. It is therefore essential that data are available to allow their identification for legislative purposes. The essential property of a food additive is a total lack of toxicity. An additive and its metabolites should be non-toxic, non-carcinogenic and non-allergenic (Hanssen 1984).

However, the main use of plant gums is in the food industry where they are used as food additives classified as stabilisers, thickeners or emulsifiers. It is now rare for a processed food not to contain a gum product (a) to correct or minimise defects in its ingredients, (b) to increase the sensory satisfaction derived and (c) to produce formulations that make possible new combinations of food ingredients (Glicksman 1975).

The analytical parameters used to characterise gums are: neutral sugar composition after mild acidic hydrolysis; ash and moisture contents; methoxyl and protein contents; specific optical rotation; intrinsic and Brookfield viscosities; equivalent weight; uronic anhydride content; amino acid composition after acidic hydrolysis; and nuclear magnetic resonance (NMR) spectra. These parameters, especially the ^{13}C NMR spectra, give a unique set of data in the form of a "fingerprint" which characterises each botanical species (Jennings and Smith 1980; Perlin and Casu 1982; Gidley 1987); and this also gives one of the most sensitive ways of establishing the identity of a botanical species. Unfortunately, NMR Spectroscopy is too expensive a technique to be generally available for commercial purposes on an every day basis and so, at least for the present, the analytical parameters of gums must be used as the means of identifying whether or not adulterants are present in commercial gums.

All experimental methods applied in this Thesis are described in Chapter 2, and in Chapter 3 of this Thesis the relationships between chemical shift values of ^{13}C NMR spectra of *Acacia* gums and the component monosaccharides, anomeric configurations, position of linkages in the overall structure of the *Acacia* gums is established by comparisons with model sugars and the known inter linkages in degraded polysaccharides.

Chapter 4 of this Thesis presents structural studies on gum arabic (derived from the defined species *Acacia senegal*), and gives the structural ^{13}C NMR "fingerprint", amino acid compositions, etc. of various fractions that were obtained.

Chapter 5 presents analytical and structural studies on the gums from *Acacia* species belonging to its Gummiferae and Vulgares Series (*A. seyal, A. sieberana, A. paolii, A. thomasii, A. leucospira, A. cheilanthifolia*), including the ^{13}C NMR spectra and amino acid compositions.

Chapter 6 presents analytical and structural studies of some of the more highly proteinaceous *Acacia* gums (*A. difficilis, A. eriopoda, A. tumida*). The ^{13}C NMR spectra and analytical data are presented for the original gums, Smith-degradation products, and fractionations separated by gel permeation chromatography. The amino acid composition of enzyme degraded *A. difficilis* gum is also reported.

Chapter 7 presents analytical and structural studies by ^{13}C NMR spectroscopy of some *Leucaena* gum exudates (*L. leucocephala, L. shannonii, L. collinsii, L. esculenta, L. diversifolia*) whose sugar and amino acid compositions after hydrolysis, and specific rotation values, are similar to that of gum arabic. The differences in the structures shows that β-L-Araf is present in most *Leucaena* gums but not in gum arabic (*A. senegal*). *Leucaena* gums are not permitted food additives; a means of their identification is therefore important.

Chapter 8 presents analytical and structural studies of *Combretum nigricans* gum by sequential Smith-degradations and mild acidic degradation. Analytical data, including amino acid compositions and ^{13}C NMR spectra of degraded *C. nigricans* gum, are presented.

Chapter 9 presents analytical studies of some gum exudates such as *Cassia grandis, Enterolobium cyclocarpum, Cercidium praecox, Lysiloma acapulcense, Senna nicaraguensis, Caesalpinia eriostachys, Parkia nitida, Prosopis flexuosa, Prosopis chilensis, Sclerocarya birrea, Pseudocedrela kotschyi, Combretum*

paniculatum, *Cassine aethiopica*, *Atalaya hemiglauca*, *Sesbania sesban*, none of which had been available for study previously.

The last Chapter presents the structural "fingerprints" of some *Acacia* gum exudates (*A. arabica, A. karroo, A. laeta, A. mellifera, A. nubica, A. polyacantha, A. robusta, A. tortilis, A. goetzii, A. wanyu,*) given by ^{13}C NMR spectra. The differences between these botanical species of the *Acacia* genus can be compared directly from the overall fine structural profiles of the gums provided by their ^{13}C NMR spectra.

Chapter 2
Experimental Methods

2.1 General Methods

Weighings: All accurate weighings were made within the range of the graticule scale (0-100 mg) of a single pan balance (Stanton Unimatic model C.L.1) having an accuracy of ±0.1 mg.

Dialysis of polysaccharides, to remove low molecular weight materials, was carried out in cellophane tubing (Medicell International Ltd., molecular weight cut off ca.1.2 x 10^4) against running tap water for 48-72 hours, unless otherwise stated.

Electrodialyses of polysaccharides were carried out in a three-compartment perspex cell fitted with cellophane membranes. The water in the outer electrode compartment was changed regularly to prevent over-heating. Electrodialysis was continued until a current (applied voltage of 300 Volts) ceased to flow.

Reductions of volume were carried out with a rotary evaporator at temperatures below 40°C, unless otherwise stated.

Moisture contents were determined by heating samples to a constant weight at 105°C.

Solubility was determined by using a centrifuge to separate the insoluble components from the gum solution for quantification after drying to constant weight.

Ash contents were determined by heating to a constant weight in a muffle furnace at 550°C.

Cationic contents were determined by Atomic Absorption Spectroscopy (Pye-Unicam SP9 Model) of the derived ash in dilute nitric acid solution.

Nitrogen contents were determined by a semi-micro Kjeldahl method.

Methoxyl contents were determined by a vapour-phase infrared method (Anderson and Duncan 1961; Anderson and Garbutt 1963). Vapour-phase infrared spectroscopy was carried out with a Perkin Elmer 137 spectrophotometer, and the peak heights obtained at 1260 cm^{-1} were compared with a calibration curve based on

known weights of methyl iodide. The methoxyl content of the polysaccharides was determined from the weight of methyl iodide produced, from the relationship

$$\%OCH_3\ (mg) = 31/142 \times CH_3I\ (mg)$$

Equivalent weight determinations on exhaustively electrodialysed polysaccharides were carried out by direct titration with standard sodium hydroxide solution (ca. 0.01 M)

$$E = W/(M \times V) \times 1000$$

where E is neutralization equivalent weight and W is the gum weight (mg); M is the molar concentration of the hydroxide solution and V is the volume (ml) of titrant required.

Uronic acid contents were calculated from the equivalent weights as 17600/E, the values being expressed as percentage of uronic anhydride rather than as uronic acid to reflects its polymeric status.

Quantitative estimation of sugars: After acidic hydrolysis of the gum, sugars were separated from hydrolysates by paper chromatography on Whatman 3MM paper. After elution from the paper, sugars were estimated colourimetrically by the phenol-sulphuric acid method (Dubois et al. 1956). The optical density was read on an Unicam SP 1300 spectrophotometer using filter 2; calibration curves were obtained from known weights of sugars. Sugar ratios were also determined by ^{13}C NMR using D_2O as solvent.

Tannin contents were estimated colourimetrically at 540 nm. Ferric chloride reacts with tannic acid to produce a blue-black colouration (FAO 1983). 0.25 ml 0.5M $FeCl_3$ was added into 10 ml 1% gum solution and a calibration curve was based on known concentrations of tannic acid.

Protein contents were determined by multiplying the nitrogen contents by a nitrogen conversion factor (NCF) which was calculated from the amino acid composition. Protein content was also determined by U.V. at 595 nm using a protein assay reagent based on Bradford method (Bradford 1976) which utilizes an absorptance shift (λ_{max}) in an acidic Coomassie Brilliant Blue G-250 solution (Pierce & Warriner Ltd.) to give the changes in the protein concentration of the eluent (Pierce and Suelter 1977). A calibration curve was obtained from known weights of protein.

2.2 Physical Methods

Specific rotations of aqueous solutions were measured using the sodium D-line with a Perkin Elmer Model 141 Polarimeter at room temperature.

Viscosity determinations (Intrinsic) were carried out in M-sodium chloride solution in an Ubelohde suspended-level dilution viscometer at 25.0±0.1°C. Solutions were filtered carefully (0.8 μm membrane filter) before additions were made to the viscometer. Flow times were measured to within 0.1 second by means of a stopwatch. The isotonic dilution technique was used. A solution of the sample (6 ml, 1%) was placed in the viscometer and the flow time was measured. Flow times were measured also for successive dilutions with M-sodium chloride solution (four additions of 2 ml each). Preliminary experiments had shown that any loss of gum from M-sodium chloride solution on filtering was negligible. Concentration values were estimated from the dry weight of gum dissolved in a known volume.

Assuming the densities of M-sodium chloride and gum solution to be equal for low concentrations of gum, the intrinsic viscosity number $[\eta]$ is calculated by the Huggins relationship:

$$\eta_{sp}/C = [\eta] + k'[\eta]^2C$$

$$[\eta] = \lim_{C\to 0} \eta_{sp}/C = \lim_{C\to 0} t\text{-}t_o/Ct_o$$

$$t\text{-}t_o/Ct_o = 1/C(t/t_o - 1) = 1/C(\eta/\eta_o - 1)$$

$$= 1/C(\eta_r - 1) = \eta_{sp}/C$$

where η_r is relative viscosity; $\eta_{sp} = \eta_r - 1$ is specific viscosity; η_{sp}/C is reduced viscosity; $[\eta]$ is intrinsic viscosity, with t_o and t being the flow times for solvent and solution respectively, and C is the concentration of gum solution (g/ml). Extrapolation of the linear plot of $t\text{-}t_o/Ct_o$ against C (when $C\to 0$) gives $[\eta]$ (Cowie 1973).

Brookfield viscosities were determined using gum solutions of concentration 25% (w/w) on a Brookfield Viscometer model RVF at speed 20 r.p.m. unless otherwise stated.

pH determinations were carried out on 25% (w/w) gum solutions using a Kent EIL 7015 pH meter at room temperature.

2.3 Chemical Methods

Acetyl contents were determined by alkaline hydrolysis followed by distillation of the acetic acid liberated, absorption in standard alkali, and back-titration with HCl (Schaltz 1965).

Crude gum (ca. 300mg) was weighed into a Kjeldahl flask and 5 ml. of 4M NaOH was added. The flask was heated with a flame until the gum was totally dissolved. The solution was transferred into the central chamber of the acetyl apparatus and H_2SO_4 (33%,5ml) was added. A fairly rapid flow of steam was passed through vigorously for 20-30 mins. to ensure quantitative transfer of acetic acid which was trapped by standard NaOH solution (ca. 0.01N). The collections were titrated with 0.01 HCl to the phenolphthalein end-point. A recovery determination was made in the same manner by using ca.10 mg β-D(+)glucose penta-acetate (formula Wt=390, theoretical acetyl content 55.1% (5 × 43 /390)) as standard. The acetyl content (formula Wt=43) of the sample was calculated from following equation:

$$\%CH_3CO = \frac{(V_{NaOH} \times N_{NaOH} - V_{HCl} \times N_{HCl}) \times 43 \times 100}{W \times 55.1}$$

where V = Volume consumption (ml.); N = Normality
W = Weight of sample (mg), 55.1% is the theoretical recovery

Sequential Smith-degradation: Smith-degradation was first developed in 1955 using gum arabic, the presence of a 1→3 linked β-D-galactan core being established thereby (Smith and Spriestersbach 1955; Goldstein et al. 1965). Anderson and co-workers conducted extensive experiments in which gum arabic and other arabinogalactan gum exudates were examined by a series of sequential Smith degradations, each degraded polymeric product serving as the starting material for the subsequent series of reaction steps (Anderson and Cree 1966; Anderson and Stoddart 1966; Anderson and Dea 1969b). Very often the yield of the ultimate Smith-degradation product was low but a great deal of information was obtained about the chains of sugar units within the gum structures. A simplified outline of the Smith-degradation procedure and its consequences was given (Stephen et al. 1990) as follows:

(a) 1,2-Diols oxidised by IO_4^-
(b) Aldehydes reduced by BH_4^-
(c) Cold hydrolysis

-Sugars so attacked and modified are released from the next sugar in chain.

-Unattacked sugars remain joined.

-Process repeated on coherent blocks of sugar units remaining.

To a 1-2% solution of the original gums, sodium metaperiodate ($NaIO_4$) solution was added to give 0.125M $NaIO_4$ concentration in the mixture. The mixture was kept at room temperature in darkness with stirring for 96 hours until the formic acid concentration amounts did not increase further; ethylene glycol (ca. 1% of the total volume) was added and the solution was dialysed against running tap water for 80 hours and then against distilled water for 12 hours. 0.25%(w/v) sodium borohydride ($NaBH_4$) was then added and, after storage for 24 hours at room temperature, the solution was dialysed against running water for 50 hours and against distilled water (regularly changed) for 18 hours; 1 N sulphuric acid was added to adjust the pH of the solution (ca. 8.5) to pH 2 followed by storage for 48 hours at room temperature with stirring. Barium carbonate was then added to neutralise (pH 7) the acidic solution which was then filtered, deionised with Amberlite IR-120(H^+) resin, and then dialysed against distilled water (regular changes) for 40 hours. The third time, distilled water was collected, vacuum evaporated and freeze-dried to give dialysate products (d-product). The solution was continuously dialysed against running tap water for a further 24 hours, and freeze-dried to give SD1 (SD-product). Subsequent Smith-degradations gave SD2, SD3, SD4.

Partial acid hydrolysis was carried out by heating the gum in 0.005M aqueous solution (pH 2) under reflux on a boiling water bath for 48 hours. After cooling, the solution was dialysed against running water, then distilled water for 2 days, then filtered and freeze-dried to obtain the degraded gum resulting from incomplete breakdown of the original gum because the stability of glycosidic linkages depends greatly on the nature of the sugar and various hemi-acetal linkages which have differing labilities towards acid. The degraded polysaccharides can serve as a useful starting point for characterisation by the standard methods applicable to the whole, natural gum.

Polysaccharide hydrolyses: Small scale polysaccharide hydrolyses were carried out with 1M sulphuric acid on a boiling water bath for 15 hours. Hydrolysates were neutralised with excess barium carbonate, filtered, deionised with Amberlite IR-120(H^+) resin, vacuum filtered, and concentrated to a syrup on a rotary evaporator.

Amino acid hydrolysis: A sufficient amount of sample to give 0.25 mg of nitrogen (1.5 mg of crude protein) was weighed and transferred quantitatively to a 50

ml round-bottomed flask (two-necked). Some anti-bumping granules and 20 ml of 6M hydrochloric acid were added and the flask was fitted to an 800 mm, air-cooled condenser. The apparatus was purged with oxygen-free nitrogen and then the contents were heated at 180°C under reflux for 20 hours under a continuous slow stream of oxygen-free nitrogen. The flask was allowed to cool and the condenser washed down with water; the solution was filtered through Whatman No.42 filter paper and evaporated to dryness at 40°C. The residue was dissolved in 10 mls citrate buffer (pH 2.2), filtered through a 0.22 μm Millipore filter, and stored frozen in a glass vial until automated analysis.

This was effected with a Rank Hilger Chromaspec Analyser as follows: A suitable aliquot (normally 60 μl) was applied to a 350×3 mm stainless steel column of cationic exchange resin (6 mm beads from Rank Hilger) and the constituent amino acids were separated by high pressure chromatography (ca. 2000lb/in^2) by elution with lithium citrate buffers of increasing ionic strength and pH. The eluted amino acids were detected by reaction with ninhydrin in a continuous flow analytical system and quantified by references to standard solutions at 570 nm (440 nm for Pro and Hyp). The amino acid composition is reported in all cases as amino acid residues per 1000 residues.

Emulsification capacity determination: A standard oil-in-water emulsification test for soluble hydrocolloids has been developed. Two aspects were considered; the ability to form an emulsion (the "Emulsifying Activity", E.A.) and subsequently to stabilise it (the "Emulsification Stability", E.S.) (James and Patel 1988; Anderson and Morrison 1989).

Limonene or paraffin oil (0.5 ml) was added to the filtered gum solution (2 ml, 5% (w/w) through Whatman No.41 filter paper) and homogenised with an Ultra-Turrax Homogeniser Model T25 for exactly 1 minute at 15,000 rpm. Immediately 0.1 ml was withdrawn from the bottom, diluted (1:250), and the absorbance measured at 500 nm with a Perkin Elmer Ultraviolet/Visible spectrometer against a blank. This absorbance gives the "Emulsifying Activity". The remaining emulsion was used to fill a syringe up to the 1.0 ml mark and allowed to stand, clamped vertically, for 30 minutes; the lower half of the emulsion (0.1 ml) was then dispersed into distilled water (25 ml). The absorbance at 500 nm of this diluted emulsion was measured against the blank. The Emulsification Stability was calculated as the absorbance of this dilution expressed relative to the Emulsification Activity:

$$\% \text{ E.S} = \frac{\text{Absorbance at 500 nm of emulsion stored for 30 min} \times 100}{\text{E.A. at 500 nm of freshly prepared emulsion}}$$

2.4 Chromatographic Separations

Paper chromatography of sugars was carried out on Whatman No.1 paper, unless otherwise stated, with the following solvent systems (v/v):

(a) ethyl acetate, acetic acid, formic acid, water (18:3:1:4)
(b) benzene, butan-1-ol, pyridine, water (1:5:3:3)
(c) ethanol, hydrochloric acid (0.1N), butan-l-ol (10:5:1)(Anderson and Munro 1969)
(d) ethyl acetate, pyridine, water (10:4:3)

Before using solvent system (c) papers were dipped in 0.3M sodium dihydrogen orthophosphate solution and air dried.

Reducing sugars were detected by spraying chromatograms with a saturated solution of aniline oxalate in ethanol/water (1:1 v/v), then heating at 105°C for about 3 minutes.

Molecular mass distribution: The molecular mass distributions of gum were monitored by gel permeation chromatography (GPC) using a glass column of dimensions 2.4 × 90 cm which was packed with Sephacryl 500 (Pharmacia) and the bed height was 80 cm. A 10 ml solution of sample was prepared and filtered through a Whatman No.1 filter paper. The solution was passed down the column under gravity. Distilled water was used and was maintained at a flow rate of 6.0 ml/hour at room temperature for eluent. A portion of the elution was monitored by the phenol-sulphuric acid method using a Corning Colorimeter Model 252 fitted with filter No.2 (430 nm) to give an absorption responding to the sugar concentrations. The eluent was collected to give fractions according to a plot of molecular mass distribution.

Molecular weight estimations: were carried out as for **Molecular mass distribution**, using Dextran Blue and *Acacia* gums of known molecular weight (established previously by the light-scattering technique) (Anderson and Dea 1969a) as standards to obtain a calibration curve.

2.5 Carbon-13 NMR Spectroscopic Methods

^{13}C NMR has proved to be one of the most efficient spectroscopic methods for configurational and conformational investigations in carbohydrate chemistry (Breitmaier and Voelter 1978). It can be utilised very effectively to determine monosaccharides qualitatively from chemical shifts, with comparisons of the chemical shifts of known sugars. The sugar composition of acid-hydrolysed polysaccharides can also be obtained directly from the intensities of the anomeric peaks of each sugar under certain operating conditions. Moreover, for di-, oligo- and polysaccharides, ^{13}C NMR spectra can supply information about the linkage positions and configurations of the constituent sugar units.

Sugar determination by ^{13}C NMR after acidic hydrolysis: Acid-hydrolysed gum in D_2O solution was put into an NMR tube. ^{13}C Fourier-transform NMR spectra at room temperature were recorded at 50.32MHz with a Bruker WP200 SY Spectrometer under standardised operating conditions suitable for sugar determinations. Tables 2-A and 2-B give the assignments of the monosaccharides frequently found after acidic hydrolysis of many plant gum exudates. In order to standardise the pre-treatment of all samples, the reference sugars were also treated with 1N H_2SO_4 in a boiling water bath for 15 hours (the hydrolysis procedure used in the analysis of natural gum exudates). The ^{13}C NMR spectra are shown in Figs. 2.1-2.8. Comparison with the spectra obtained for standard sugars that had not however been refluxed with acid show that most neutral sugars are indeed stable under these analytical conditions except for L-arabinofuranose, which is converted to L-arabinopyranose. But acidic sugars, especially glucuronic acid, are less stable; Figs. 2.7(b) and 2.8(b) show the major extra peaks (marked x) which do not appear in the spectra before the acidic-hydrolysis treatment (Figs. 2.7(a) and 2.8(a)).

The chemical shifts of the different carbon atoms in sugars have been reported by several authors (Breitmaier and Voelter 1978; Jarrell et al. 1979; Reuben 1984; Pinto 1991; Juhngon et al. 1972; Breitmaier et al. 1979). The shifts depend on the operating conditions used, e.g. type of spectrometer, solvent, temperature, pH and so on. Differences of up to 2 ppm appear, from these publications, to arise from the use of different operating conditions. The chemical shifts listed in Tables 2-A and -B are based on the operating conditions adopted for all NMR spectra reported in this Thesis.

^{13}C-NMR spectra for natural gums were obtained for ca. 10% gum solutions (depending on the resulting viscosity) in D_2O at room temperature at 50.32 MHz with a Bruker WP200 spectrometer (overnight runs of approximate 12 hours). The intensities and integrals quoted for the ^{13}C NMR spectra are relative values.

Table 2-A ^{13}C NMR. assignments of chemical shifts(ppm) for sugars in D_2O

Sugars	C_1	C_2	C_3	C_4	C_5	C_6
β-D-Galp	96.50	72.00	72.86	68.83	75.14	61.08
α-D-Galp	92.34	69.39	68.45	69.27	70.50	61.27
β-L-Araf	95.10	76.18	73.97	81.29	61.15	
α-L-Araf	101.03	81.38	75.57	82.95	60.98	
β-L-Arap	92.52	68.64	68.64	68.43	62.43	
α-L-Arap	96.70	71.84	72.40	68.43	66.33	
β-L-Rham	93.45	71.30	72.71	71.96	71.81	16.90
α-L-Rham	93.92	70.78	69.95	72.71	68.21	16.90
β-D-Xylp	96.50	73.92	75.71	69.12	65.07	
α-D-Xylp	92.11	71.35	72.73	69.30	60.82	
β-D-Manp	93.59	71.16	72.96	66.52	76.02	60.90
α-D-Manp	93.94	70.62	70.17	66.77	72.27	60.90
β-D-Glup	95.79	74.02	75.64	69.49	75.80	60.66
α-D-Glup	91.98	72.67	71.35	69.49	71.31	60.51
β-D-GlupA	95.72	73.35	74.07	70.72	74.95	172.02
α-D-GlupA	92.00	72.08	71.13	70.06	70.92	172.97
β-D-GalpA	95.86	70.99	71.98	68.36	73.67	172.64
α-D-GalpA	92.07	69.87	67.53	69.37	69.86	171.72
*β-D-Frucf	66.08	99.74	69.51	71.62	70.92	64.79
*α-D-Frucf	65.44	105.84	84.62	77.41	82.60	62.79
*β-L-Fuco	96.30	71.80	73.00	71.40	70.50	15.60
*α-L-Fuco	92.20	69.40	68.20	71.80	66.00	15.60

* Quoted from reference (Breitmaier and Voelter 1978).

Table 2-B ^{13}C NMR assignments of chemical shifts(ppm) for sugars in D_2O

δ(ppm)	Carbon	δ(ppm)	Carbon	δ(ppm)	Carbon
16.90	α-Rham 6	70.72	β-GlupA 4	81.29	β-Araf 4
16.90	β-Rham 6	70.72	α-GlupA 5	81.38	α-Araf 2
60.98	α-Araf 5	70.78	α-Rham 2	82.95	α-Araf 4
61.08	β-Gal 6	71.13	α-GlupA 3	92.03	α-GlupA 1
61.15	β-Araf 5	71.30	β-Rham 2	92.34	α-Gal 1
61.27	α-Gal 6	71.81	β-Rham 5	92.52	β-Arap 1
62.43	β-Arap 5	71.84	α-Arap 2	93.45	β-Rham 1
66.33	α-Arap 5	71.96	β-Rham 4	93.92	α-Rham 1
*67.55	α-GalpA 3	72.00	β-Gal 2	95.10	β-Araf 1
68.21	α-Rham 5	72.08	α-GlupA 2	95.77	β-GlupA 1
68.43	β-Arap 4	72.17	α-Rham 4	96.50	β-Gal 1
68.43	α-Arap 4	72.40	α-Arap 3	96.70	α-Arap 1
68.45	α-Gal 3	72.71	β-Rham 3	101.03	α-Araf 1
68.64	β-Arap 2	72.86	β-Gal 3	172.02	β-GlupA 6
68.64	β-Arap 3	73.35	β-GlupA 2	172.97	α-GlupA 6
68.83	β-Gal 4	73.97	β-Araf 3		
69.27	α-Gal 4	74.07	β-GlupA 3		
69.39	α-Gal 2	74.95	β-GlupA 5		
69.95	α-Rham 3	75.14	β-Gal 5		
70.06	α-GlupA 4	75.57	α-Araf 3		
70.50	α-Gal 5	76.18	β-Araf 2		

* This is an identical peak for galacturonic acid.

The integral values are more accurate than intensities to represent the amounts of the carbons; although both intensities and integrals were given by the spectrometer for the earliest spectra obtained in this study, only intensities were quoted on those obtained at a later stage.

SUGARS	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Galp	96.50	72.00	72.86	68.83	75.14	61.08
Intensity	22	21	21	21	25	22
α-D-Galp	92.34	69.39	68.45	69.27	70.50	61.27
Intensity	11	12	12	14	12	12

*The intensities indicate that the equilibrium mixture contains ca.33% α- and 67% β-D-Galactose.

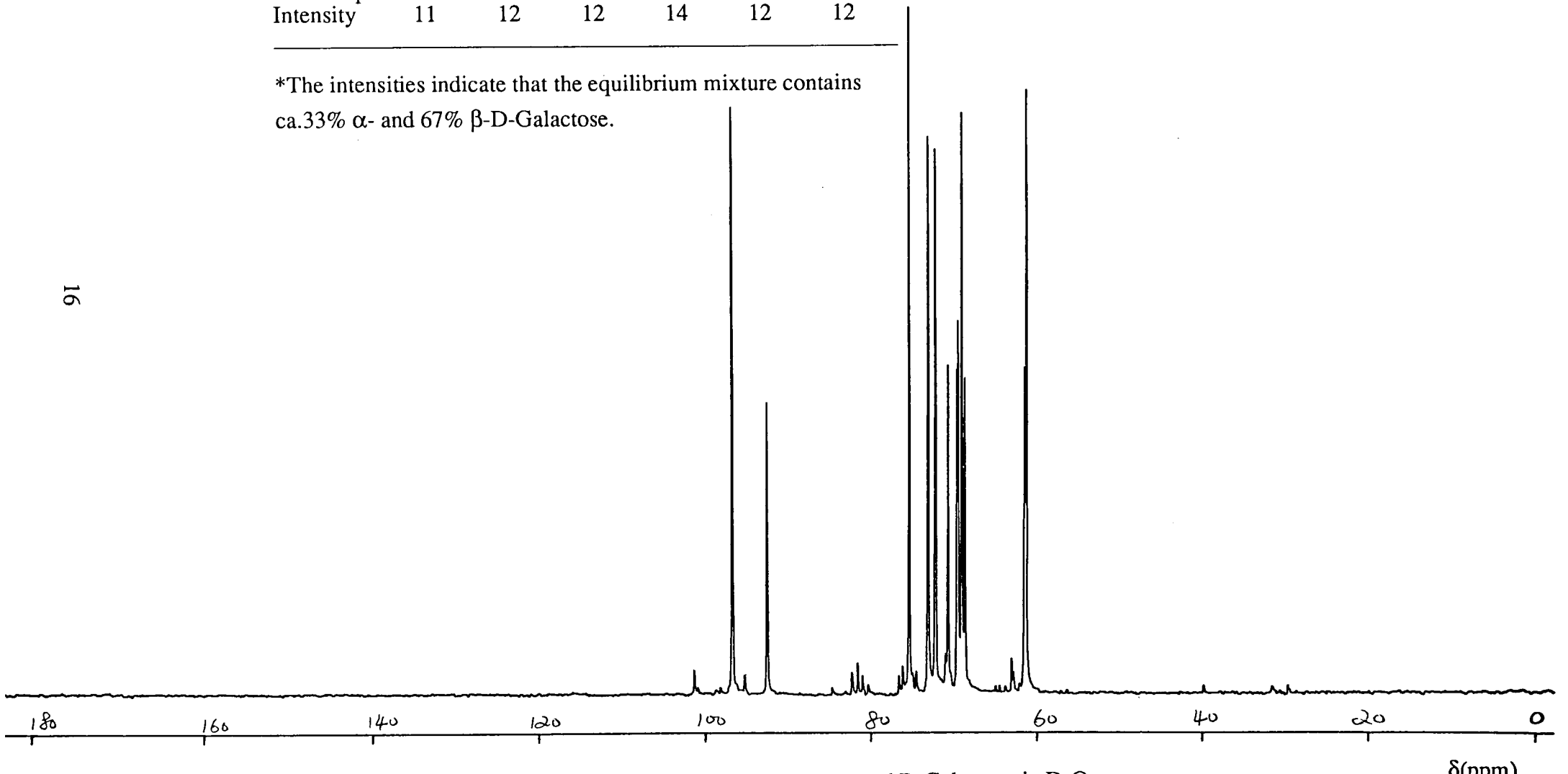

Fig. 2.1 ^{13}C NMR spectrum for acid-treated D-Galactose in D_2O

SUGARS	C-1	C-2	C-3	C-4	C-5
β-L-Arap	92.52	68.64	68.64	68.43	62.43
Intensity	9	12[a]	12[a]	25[b]	8
α-L-Arap	96.70	71.84	72.40	68.43	66.33
Intensity	18	18	17	25[b]	16
β-L-Araf	95.10	76.18	73.97	81.29	61.15
Intensity	0.8	0.9	1.0	1.2	1.7
α-L-Araf	101.03	81.38	75.57	82.95	60.98
Intensity	1.3	1.5	1.4	1.4	1.1

[a,b] peaks overlap.

*The intensities indicate that the equilibrium mixture contains ca.67% α- and 33% β-L-Arabinopyranose and ca. 62% α- and 38% β-L-Arabinofuranose.

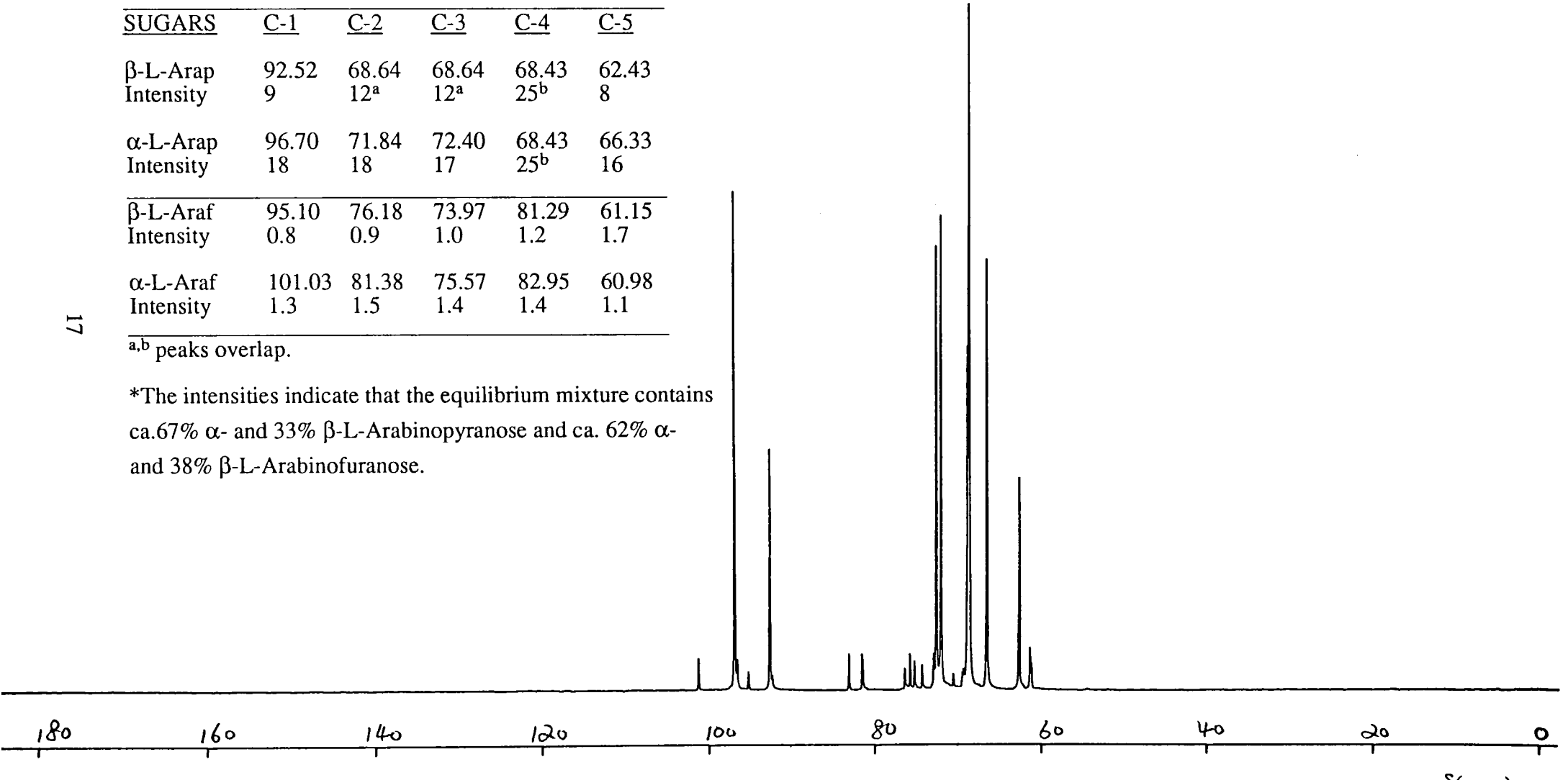

Fig. 2.2 ^{13}C NMR spectrum for acid-treated L-Arabinose in D_2O

SUGARS	C-1	C-2	C-3	C-4	C-5	C-6
β-L-Rham	93.45	71.30	72.71	71.96	71.81	16.90
Intensity	10	10	10	15	15	25[a]
α-L-Rham	93.92	70.78	69.95	72.17	68.21	16.90
Intensity	17	16	17	18	18	25[a]

[a] peaks overlap.

*The intensities indicate that the equilibrium mixture contains ca.62% α- and 38% β-L-Rhamnose.

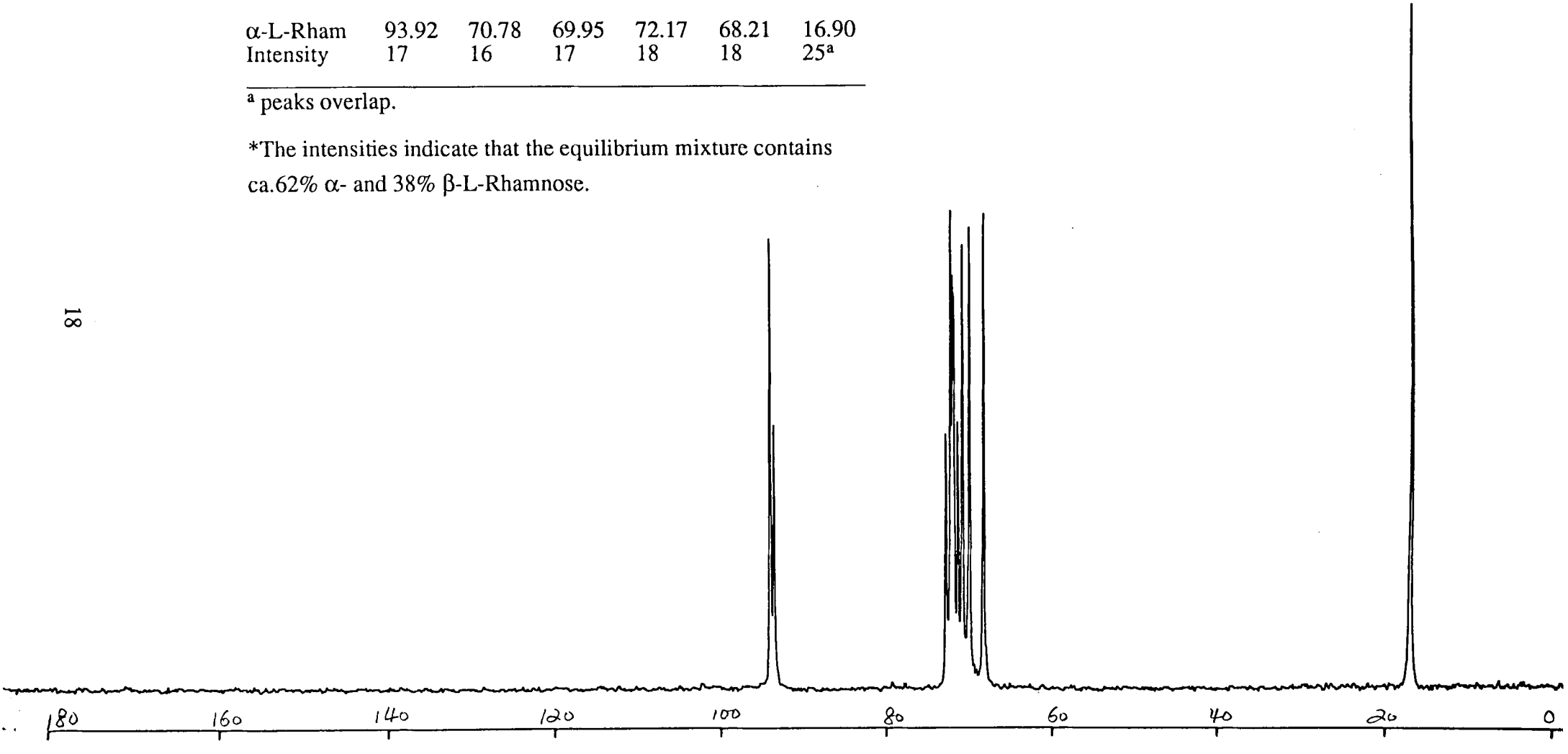

Fig. 2.3 ^{13}C NMR spectrum for acid-treated L-Rhamnose in D_2O

SUGARS	C-1	C-2	C-3	C-4	C-5
β-D-Xylp	96.50	73.92	75.71	69.12	65.07
Intensity	23	22	22	23	25
α-D-Xylp	92.11	71.35	72.73	69.30	60.82
Intensity	14	13	13	15	13

*The intensities indicate that the equilibrium mixture contains ca.37% α- and 63% β-D-xylose.

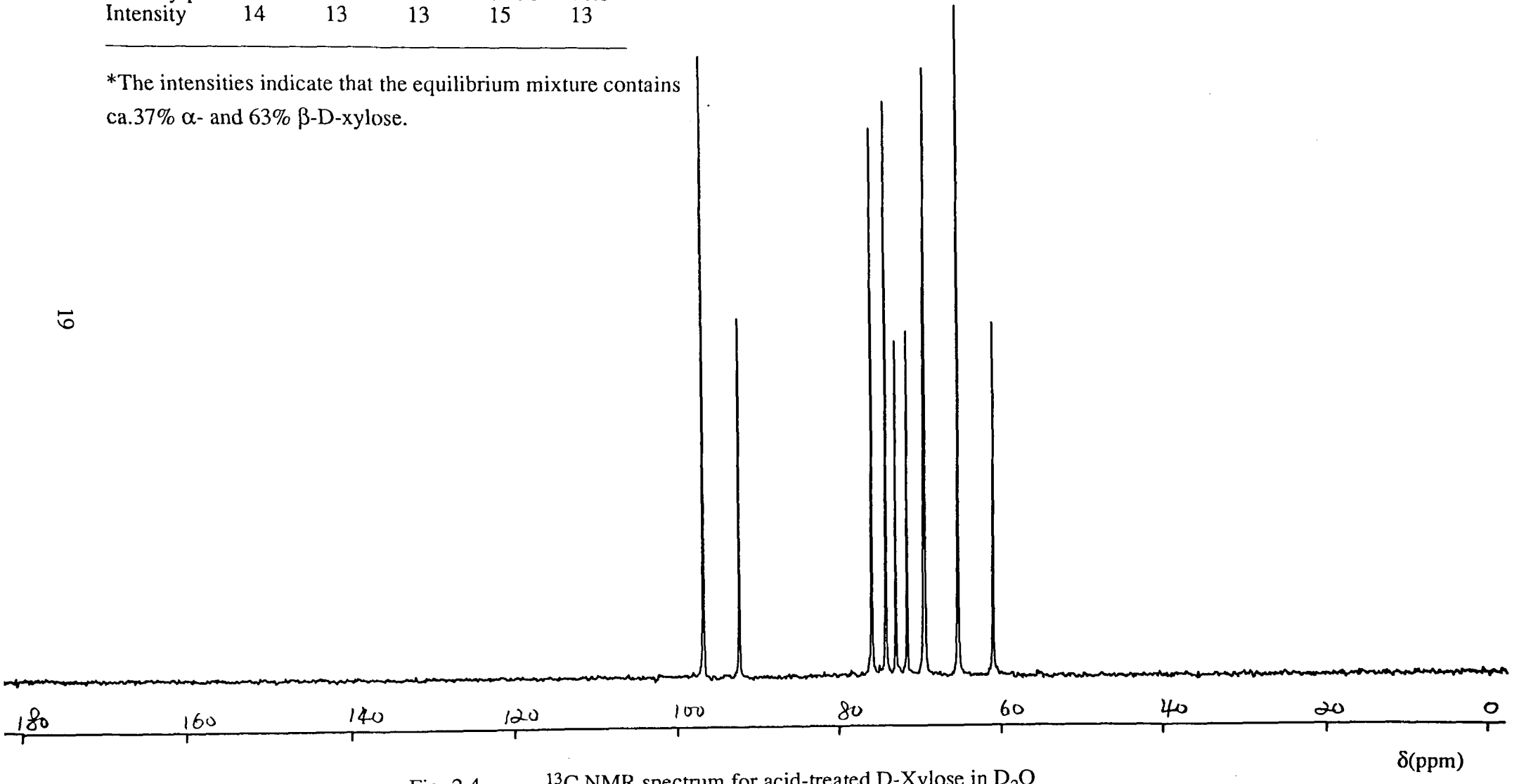

Fig. 2.4 ^{13}C NMR spectrum for acid-treated D-Xylose in D_2O

SUGARS	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Manp	93.59	71.16	72.96	66.52	76.02	60.90
Intensity	9	9	8	8	9	25[a]
α-D-Manp	93.94	70.62	70.17	66.77	72.27	60.90
Intensity	18	16	18	18	17	25[a]

[a] peaks overlap.

*The intensities indicate that the equilibrium mixture contains ca. 67% α- and 33% β-D-Mannose.

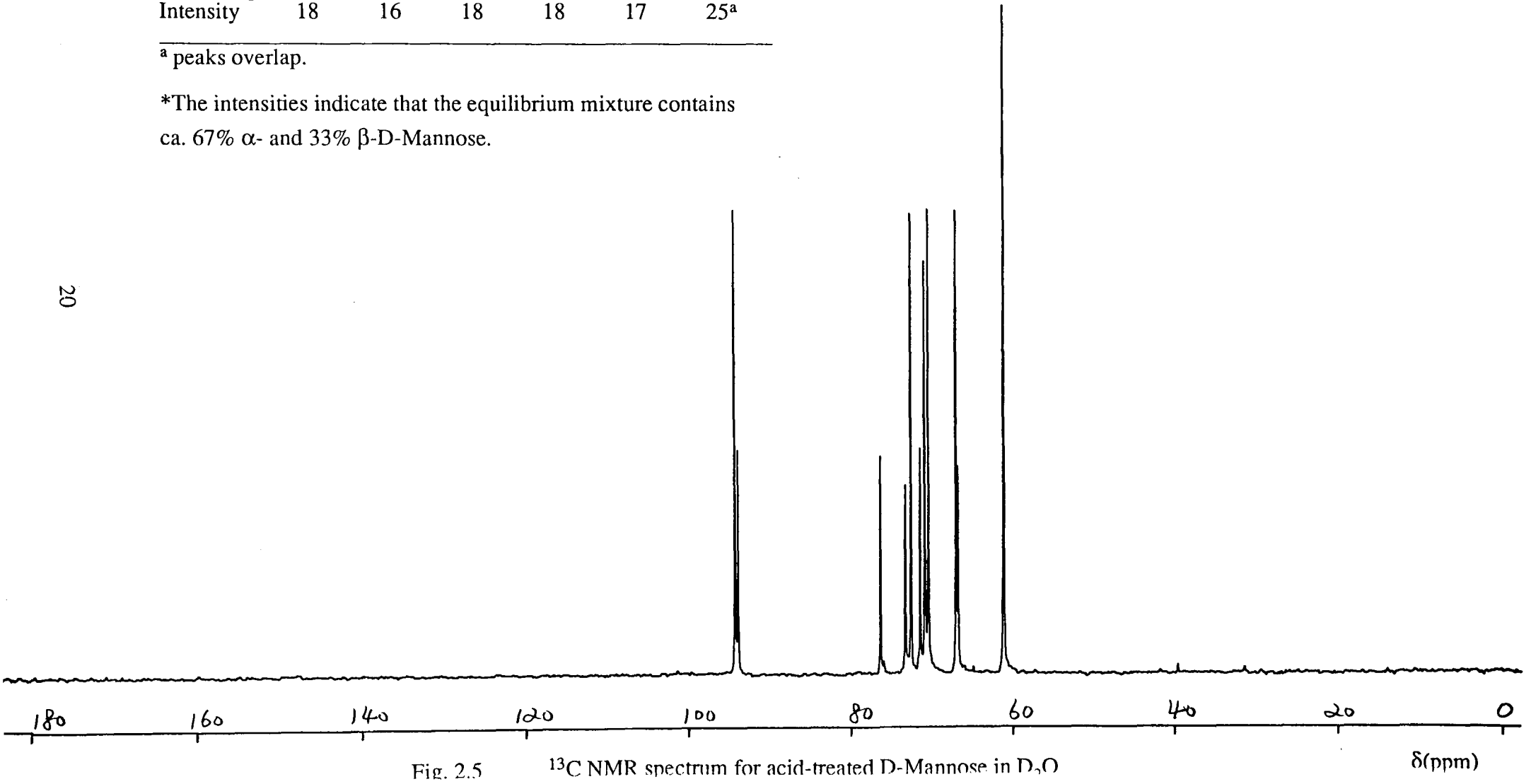

Fig. 2.5 ^{13}C NMR spectrum for acid-treated D-Mannose in D_2O

SUGARS	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Glup	95.79	74.02	75.64	69.49	75.80	60.66
Intensity	20	23	23	25[a]	24	21
α-D-Glup	91.98	72.67	71.35	69.49	71.31	60.51
Intensity	14	13	16	25[a]	17	13

[a] peaks overlap.

*The intensities indicate that the equilibrium contains ca. 40% α- and 60% β-D-Glucose.

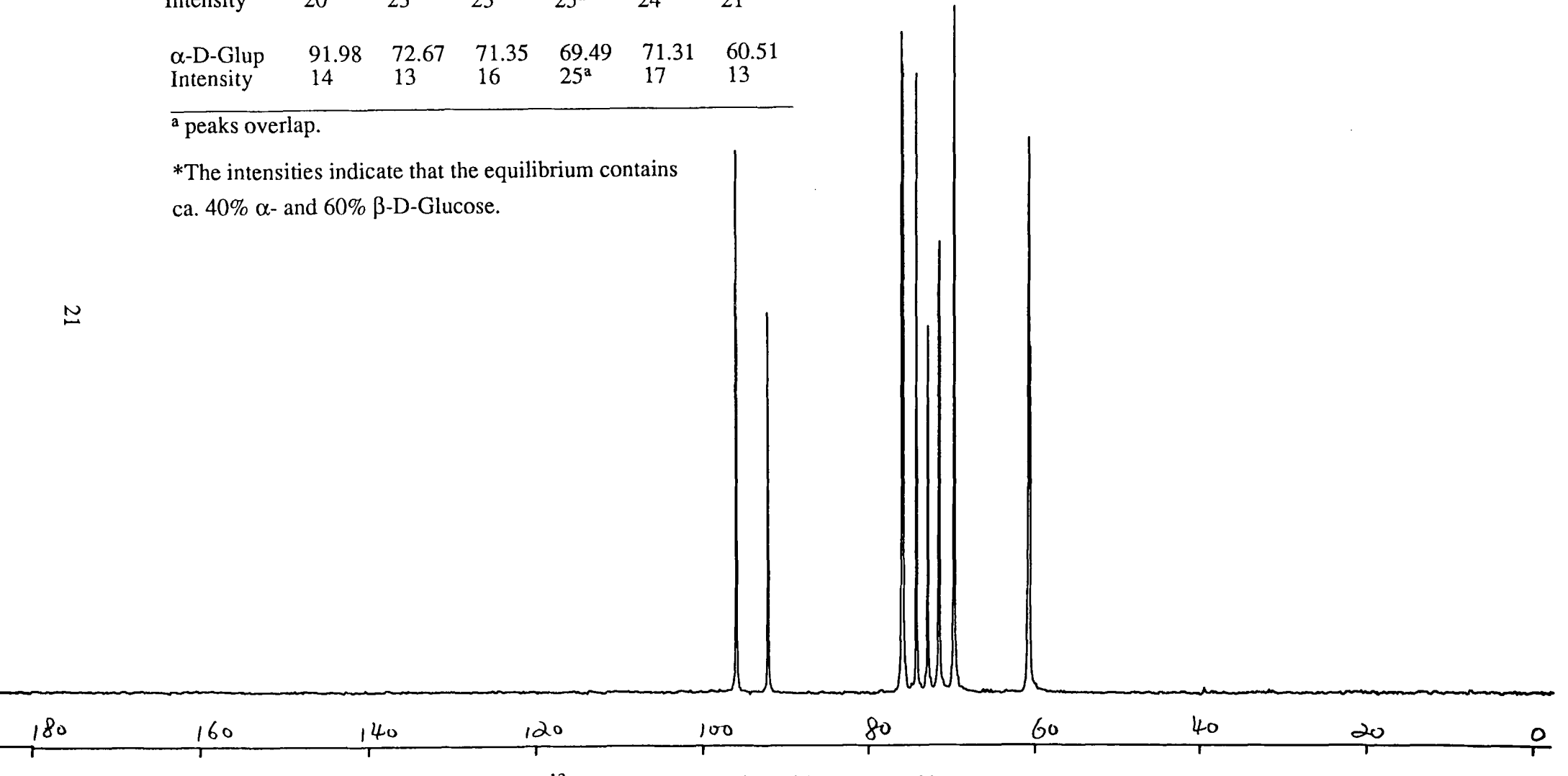

Fig. 2.6 ^{13}C NMR spectrum for acid-treated D-Glucose in D_2O

SUGARS	C-1	C-2	C-3	C-4	C-5	C-6
β-D-GlupA	95.72	73.35	74.07	70.72	74.95	172.02
Intensity	23	24	22	23	24	4
α-D-GlupA	92.00	72.08	71.13	70.06	70.92	172.97
Intensity	22	22	23	21	25	4

*The intensities indicate that the equilibrium mixture contains ca. 49% α- and 51% β-D-Glucuronic acid.

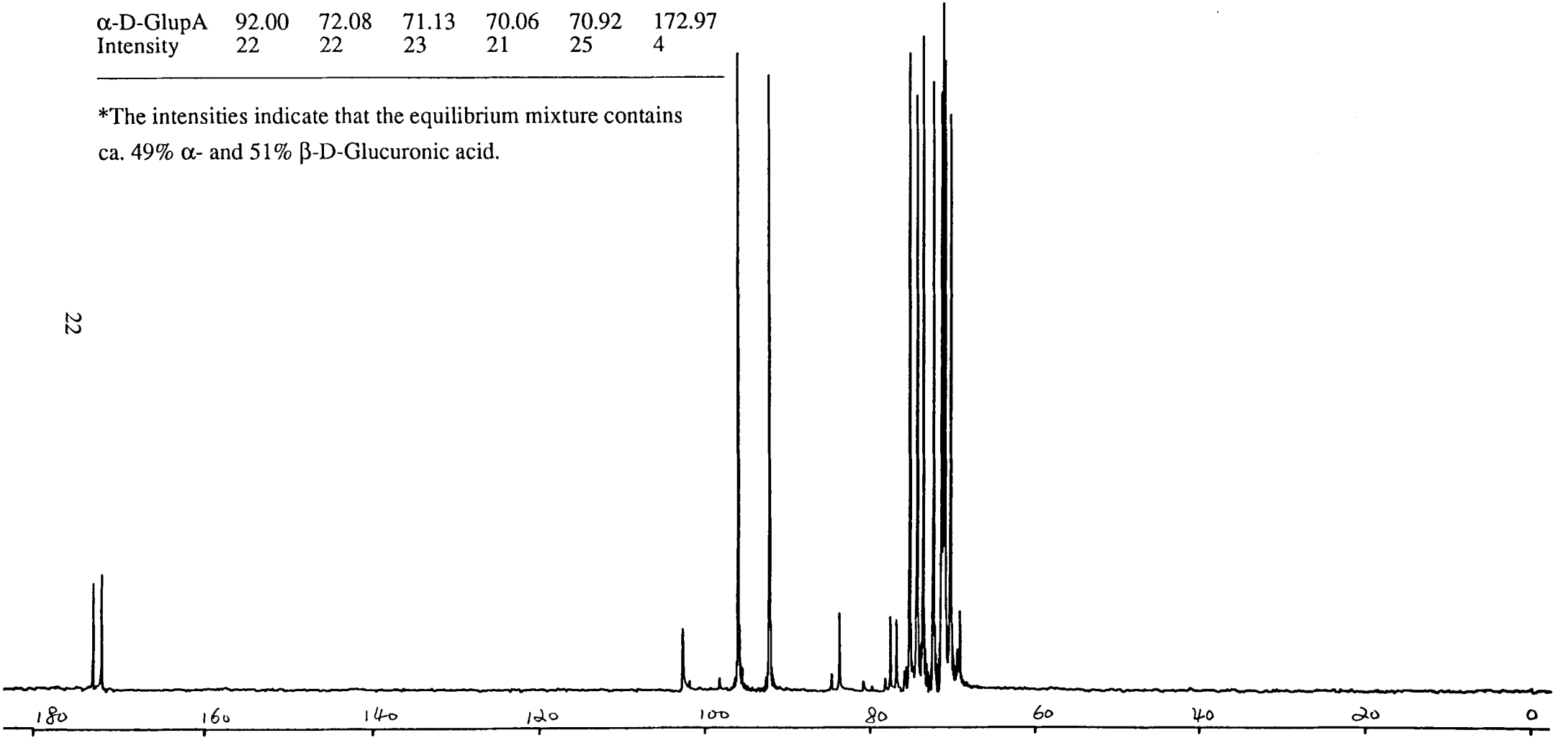

Fig. 2.7(a) ^{13}C NMR spectrum for D-Glucuronic acid (no acidic pretreatment) in D_2O

Peaks appearing after
the acidic pretreatment
shown x; see Fig. 2.7(a)

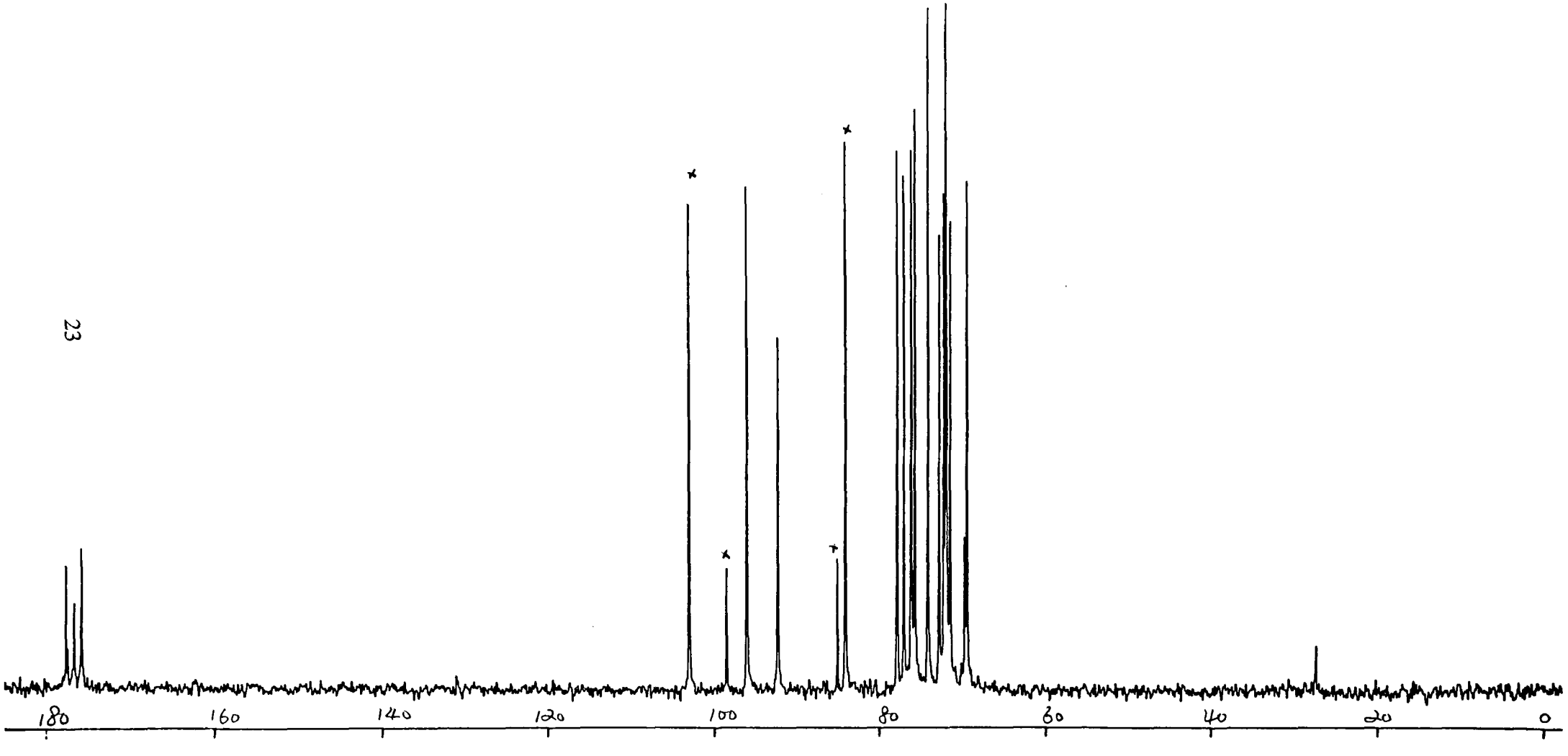

Fig. 2.7(b) ^{13}C NMR spectrum for acid-treated D-Glucuronic acid in D_2O

SUGARS	C-1	C-2	C-3	C-4	C-5	C-6
β-D-GalpA	95.86	70.99	71.98	68.36	73.67	172.64
Intensity	19	20	20	18	18	2.5
α-D-GalpA	92.07	69.87	67.53	69.37	69.86	171.72
Intensity	17	25[a]	18	21	25[a]	1.8

[a] peaks overlap.

*The intensities indicate that the equilibrium mixture contains ca. 46% α- and 54% β-D-Galacturonic acid.

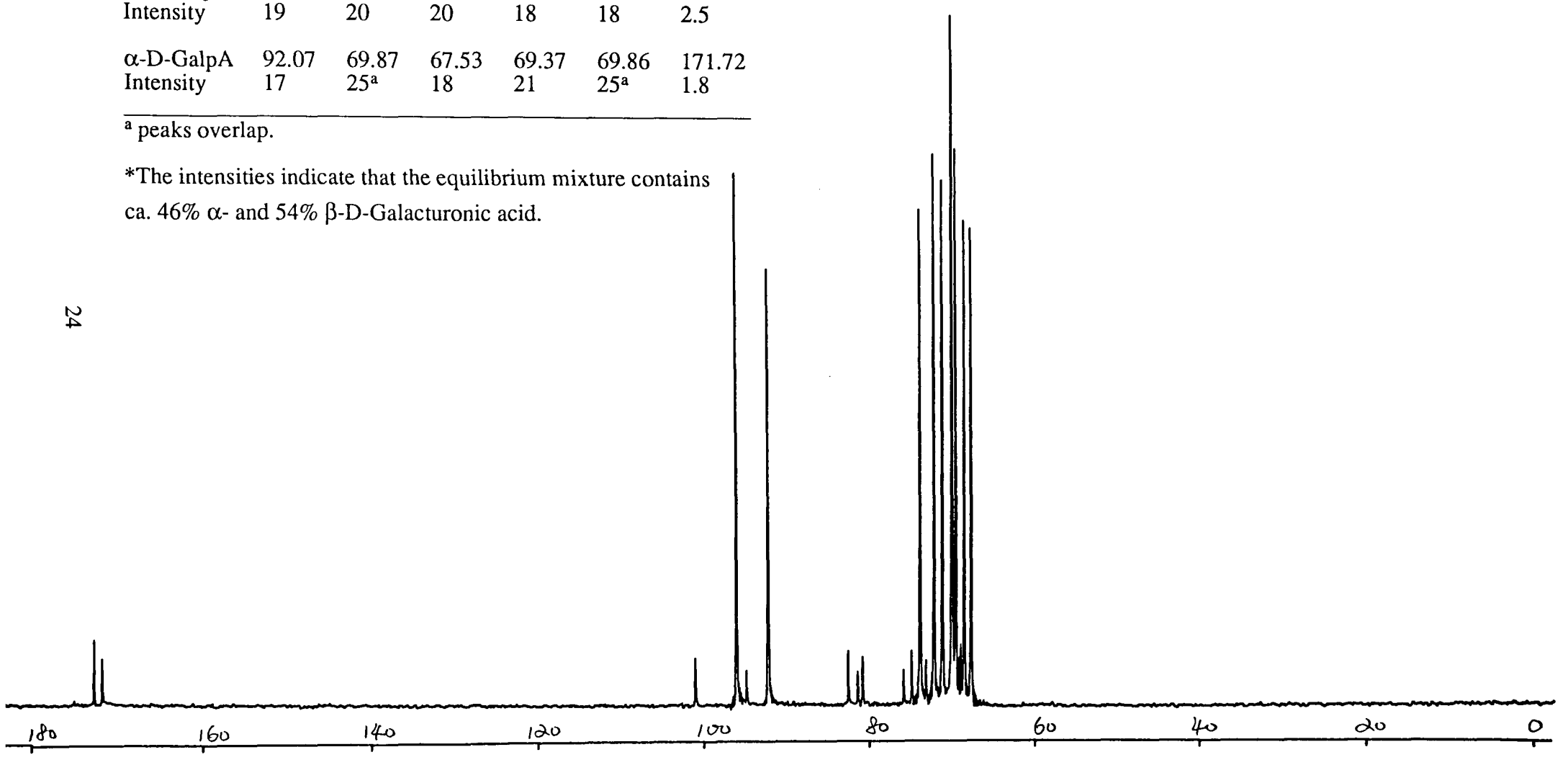

Fig. 2.8(a) ^{13}C NMR spectrum for D-Galacturonic acid (no acidic pretreatment) in D_2O

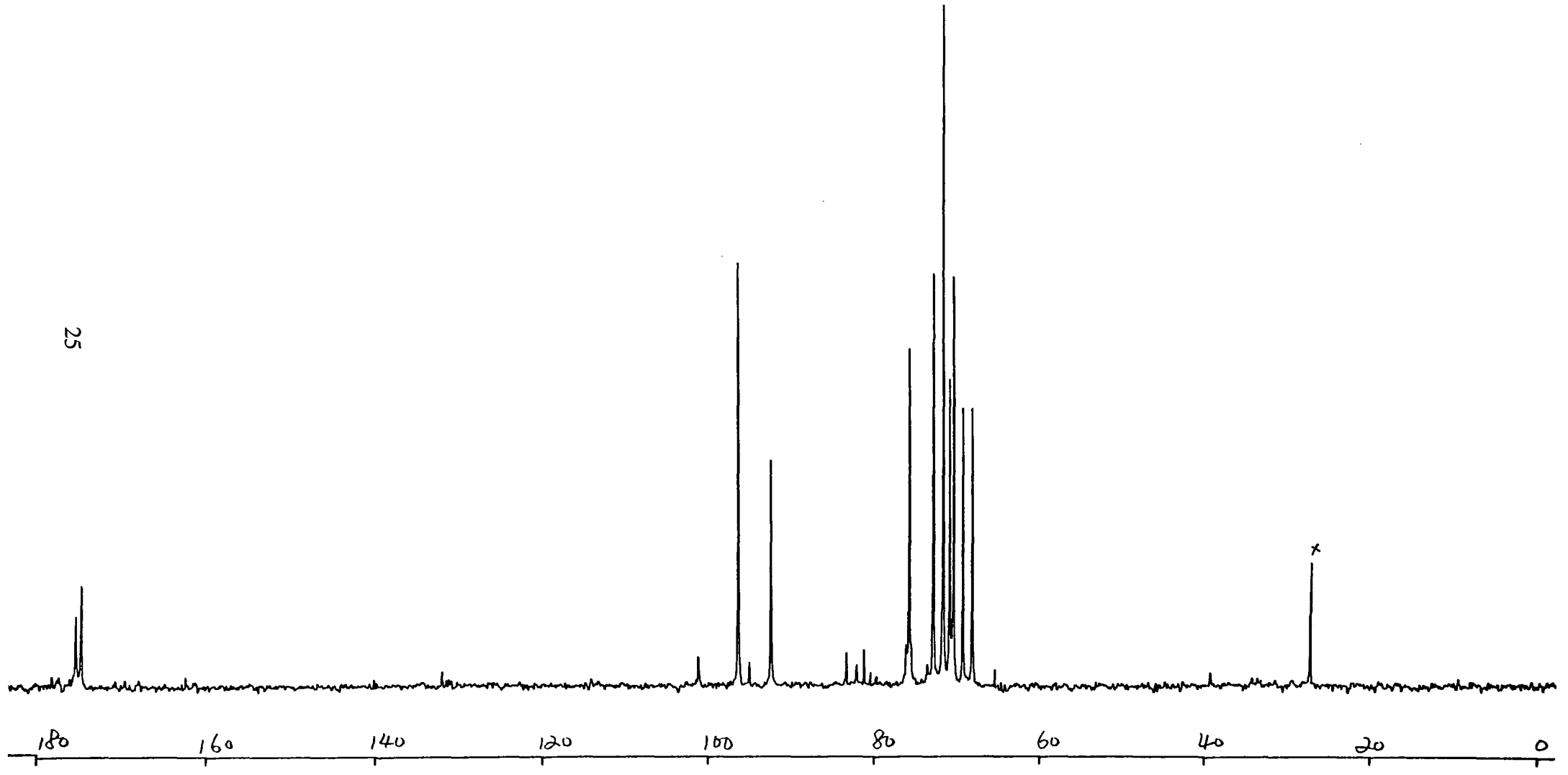

Fig. 2.8(b) ^{13}C NMR spectrum for acid-treated D-Galacturonic acid in D_2O

Chapter 3
Gum Structural Studies by the Carbon-13 NMR Method

3.1 Introduction

Carbon-13 nuclear magnetic resonance has proved to be a powerful tool for studies of biopolymers in general and has been established as a valuable technique in the structural determination of polysaccharides (Jennings and Smith 1980). It has also been applied in a variety of ways to purified oligosaccharides, isolated polysaccharides, partially hydrolysed and whole natural tree exudates to obtain structural information on anomeric configurations, linkages, and branching arrangements. In comparison with proton NMR spectroscopy, it gives better signal separation due to the wider range of chemical shifts involved (Stothers 1972). The technique is rapid, non-destructive and can be used on relatively small amounts of polysaccharides (100 to 200 mg). In general, the most informative method of analysis is based on the correlation of the chemical shifts of the carbon atoms of the polysaccharide units. Related monosaccharides, substituted monosaccharides and polysaccharides can be employed as model compounds. Experience and literature searches indicate that the chemical shifts of monosaccharides are similar to those of the monosaccharide units within a polysaccharide except for substituent effects. The effects produced by the attachment of any substituent to a sugar moiety cause an increase in the chemical shift (from 5 to 10 ppm normally, but it also depends on the linkage environment) of the carbon directly involved in the linkage. This is usually accompanied by a decrease of small magnitude (but sometimes by an increase, normally less than 2 ppm) in the chemical shifts of the neighbouring β-carbon atom and γ-carbon atom. The effects decrease as the inter-atomic distance increases. Thus, the differences in chemical shift between monosaccharide and polysaccharide can be used to determine the position of linkages. Moreover, similarities in chemical shifts, especially on selective carbon atoms known to be sensitive to change in anomeric configuration, can be employed to determine the configuration of linkages.

Additional information on the overall structural arrangement of the polysaccharide can be obtained by an examination of the ^{13}C-line-width. Narrow peaks are usually given by the sugar units which are terminal groups in side chains; while broader peaks indicate more restricted segmental motion or a greater variety of chemical environments such as often occur in the backbone of a polysaccharide

(Selvendran and Ryden 1990). The integral of the assignment indicates roughly the relative amounts of the different sugar units, although the chemical environments and the flexibilities of the sugar units affect the assignment signal's strength. The chemical shifts of a monosaccharide are, however, slightly dependent on the different solvents; solution pH; temperature (Meldal and Christiansen 1991); and operating experimental parameters. Although the assignment data of ^{13}C NMR chemical shifts for monosaccharides and several oligosaccharides were reported by several authors (Breitmaier and Voelter 1978; Bock and Thogersen 1982; Bock et al. 1984; Whistler 1980), the chemical shifts quoted by these authors differ slightly. In order to obtain accurate information it is necessary to obtain the spectra for standard sugars under the same conditions as for the polysaccharide hydrolysates.

3.2 Polysaccharide Structure and Linkage Determination

3.2.1 Di-saccharide Linkages

Many ^{13}C NMR studies on O-methylated sugars have been reported (Yoshio et al. 1978; Hamer and Perlin 1976; Pinto 1991; Defaye and Wong 1986; Gorin and Mazurek 1975; Mizutani et al. 1989; Wagner and Jordan 1988; Utille and Kovac 1986). A large amount of carbon-13 NMR data for most of the commonly occurring disaccharides have also been summarised (Hoffman et al. 1986; Small and McIntyre 1989). Although the chemical shift values reported varied slightly, it is evident that a substituent MeO-carbon causes a downfield shift for the resonance of 5~10 ppm, and the signals of the neighbouring carbons are generally shifted slightly by ~1ppm. Differences in linkages and in neighbouring environments also affect the assignment values.

The ^{13}C NMR spectra of monosaccharides exhibit three main chemical shift regions. These result from the signals for anomeric (92~102 ppm), methylene (60~67 ppm) and the other methine (68~85 ppm) carbons. Within each of these regions, unequivocal assignments may be made by considering the isotopic multiplets. But the spectra of di- or poly- saccharides normally contain four main chemical shift regions, i.e. 92~110 ppm, 79~87 ppm, 68~78 ppm and 60~68 ppm. Two other unambiguous regions at extreme fields can be assigned viz. 172~175 ppm for -COOH or -C=O groups and 15~23 ppm for C-$\underline{C}H_3$. In addition, ~57 ppm (-OMe), 20~40 for C-$\underline{C}H_2$-C may occur. Table 3.1 lists some assignments of possible sugar unit linkages.

Table 3.1(a) ^{13}C NMR assignments of sugar linkages in *Acacia* gums (±1 ppm depending upon the linkage environments)

C linked	C_1	C_2	C_3	C_4	C_5	C_6
α-D-Galp	92.34	69.39	68.45	69.27	70.50	61.27
1,α-Galp	100.03	68.5	68.7	70.0	71.3	61.2
β-D-Galp	96.50	72.00	72.86	68.83	75.14	61.08
1,β-Galp	103.5	70.8	72.8	69.3	75.1	61.0
	103.0	70.2		68.4		
1,3,Galp	103.4	70.3	81.4	68.6	75.1	61.0
1,6,Galp	103.4	70.8	72.8	69.3	73.7	69.3
1,3,6,Galp	103.7	70.3	81.4	68.6	73.7	69.3
			82.0			
1,3,4,6,Gp	103.7	70.3	81.4	74.3	73.4	69.3
α-L-Araf	101.03	81.38	75.57	82.95	60.98	
1,α-Araf	109.4	80.3	76.6	83.8	61.1	
1,3,α-Araf	108.3		84.8	83.0	61.1	
1,5,α-Araf	108.3	80.4	76.6	83.6	68.6	
Af→Af	107.4	81.2	76.6	84.0	61.1	
1,2,Af		87.7				
β-L-Araf	95.10	76.18	73.97	81.29	61.15	
1,β-Araf	101.5	76.2	72.9	81.9	63.3	
1,2,β-Araf	99.7	83.9	72.9	81.9	63.1	
	99.9					
1,3,β-Araf	101.4	76.2	82.7	81.9	62.9	
β-L-Arap	92.52	68.64	68.64	68.43	62.43	
1,β-Arap	99.2	70.8	71.9	71.9	63.0	
1,4,Arap	97.6	69.6	72.6	81.2	62.0	
1,2,Arap	97.6	81.8	72.6	71.7	61.7	
1,2,3,Ap	97.0	82.4	78.6	73.1	62.3	
	96.7				63.3	
α-L-Arap	96.70	71.84	72.40	68.43	66.33	
1,α-Arap	104.3		72.2		67.2	
α-L-Rham	93.92	70.78	69.95	72.71	68.21	16.90
1,α-Rham	100.6	70.7	70.1	72.0	68.6	16.6
β-L-Rham	93.45	71.30	72.71	71.96	71.81	16.9
1,β-Rham	100.6	70.7	72.6	72.0	72.2	16.6
		70.2	70.2			
β-GlupA	95.72	73.35	74.07	70.72	74.95	172.02
1,β-	102.5	73.4	74.1	70.7	76.2	175.0
1,4,β-	102.5	73.4	74.1	79.1	76.2	175.0
α-GlupA	92.00	72.08	71.13	70.06	70.92	172.97
1,4,α-	100.0	72.2	73.3	82.7	71.3	173

* $-OCH_3$ group in 4-O-Me-β-D-GlupA occurs at 59.8-60.1 ppm whereas $-OCH_3$ in 4-O-Me-α-D-GlupA occurs at 57.5 ppm. Acetyl groups occur around 20-23 ppm.

Table 3.1(b) The composition of sugar units indicated by the anomeric peaks in ^{13}C NMR spectra of *Acacia* gums

Chemical shift (ppm)	C_1 of
110.0 - 109.3	α-L-Araf(1→
108.5 -108.3	→α-L-Araf(1→
107.5 -107.2	→α-L-Araf(1→Ara(1→ (with 61.1 -61.4 ppm from C_5)
104.5 -104.1	α-L-Arap(1→ (with 67.8 -67.0 ppm from C_5)
<104.0	→α-L-Arap(1→ (with 67 -66 ppm from C_5)
103.9 -102.9	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.1 -61.4 ppm from C_6)
102.9 -102.4	→β-D-GlupA(1→ and β-D-GlupA(1→
101.5 -101.3	→3)β-L-Araf(1→ or β-L-Araf(1→ (with 63.2 -62.8 ppm from C_5)
100.8 -100.4	α-L-Rham(1→ (with 16.6 -16.4 ppm from C_6)
100.0	→α-D-Gal(1→ and α-D-Gal(1→ →α-D-GlupA(1→ and α-D-GlupA(1→
100 -99.4	→2)β-L-Araf(1→ (with 63.2 -62.8 ppm from C_5)
100 -99.2	β-L-Arap(1→ (with 63.1 -62.9 ppm from C_5)
99.4 -97.6	→)β-L-Arap(1→ (with 63 -62 ppm from C_5)
97.7 -97.0	→2)β-L-Arap(1→ (with 61.6 -62.0 ppm from C_5)
97.0 -96.7	→2,3)β-L-Arap(1→ (with 62.3 -63.3 ppm from C_5)
60.1 -59.8	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA

The anomeric regions are very useful for identifying the sugars present in polysaccharides. The linkage positions can be deduced by carefully comparing the

other carbon's chemical shifts with those for single sugars, bearing in mind that different linkages and different carbon positions may affect the chemical shift (normally by less than 2 ppm). For example, if galactopyranose and arabinofuranose are indicated in the polysaccharide from the anomeric region of the spectrum, but there are no resonances around 60~62 ppm, then it is apparent that the galactose 1,6 linkage and the arabinose 1,5 linkage are involved. Other important diagnostic regions are 78~86 ppm, within which range most 2,3,4,(5) linked carbons give signals; and peaks at 109 ppm and 108 ppm usually represent furanoside C_1 linkages (both arabinofuranose and galactofuranose).

3.2.2 Structure Determination of Polysaccharide

Figs. 3.1 and 3.2 show the ^{13}C NMR spectra for gum arabic (*Acacia senegal*) after 6 and 7 successive Smith-degradations. Sugar analyses by paper chromatography indicated that rhamnose and glucuronic acid were absent and only galactose and arabinose were left in the core in the ratios 80:20 and 91:9 respectively. These ratio changes were also confirmed by ^{13}C NMR sugar spectra. Fig. 3.1 and Fig. 3.2 themselves also show such structural changes in broad agreement with a previous report (Churms et al. 1983) as supported by ^{13}C NMR studies (Defaye and Wong 1986). Tables 3.2 and 3.3 list the possible interpretations of the signals given by the gum arabic core since a much simpler structure than the original polysaccharide is reached after Smith-degradations. Although some peaks still remain overlapped, and may be reversed within the 68~75 ppm region (Defaye and Wong 1986), the other regions are unambiguous and allow identification of the possible linkages. These two spectra show the structural changes from Araf→1,3,6,Gal→ and Gal→1,3,6,Gal→ (at the 6th Smith-degradation stage), to the mainly linear (1→3)β-D-galactan structure in the final core of gum arabic at the 7th stage.

The integral of the assignments of a ^{13}C NMR spectrum indicate the relative amounts of certain carbon atoms in the polysaccharide and this can be used to give a rough estimate of the sugar composition.

ppm	Intensity
109.24	14.4
103.72	12.8
83.92	13.9
81.30	16.8
80.29	8.2
76.53	15.5
75.51	4.2
74.90	12.1
73.49	9.6
72.82	8.8
70.81	10.9
70.18	13.8
69.23	19.9
68.70	21.7
61.14	22.6
61.02	25.0

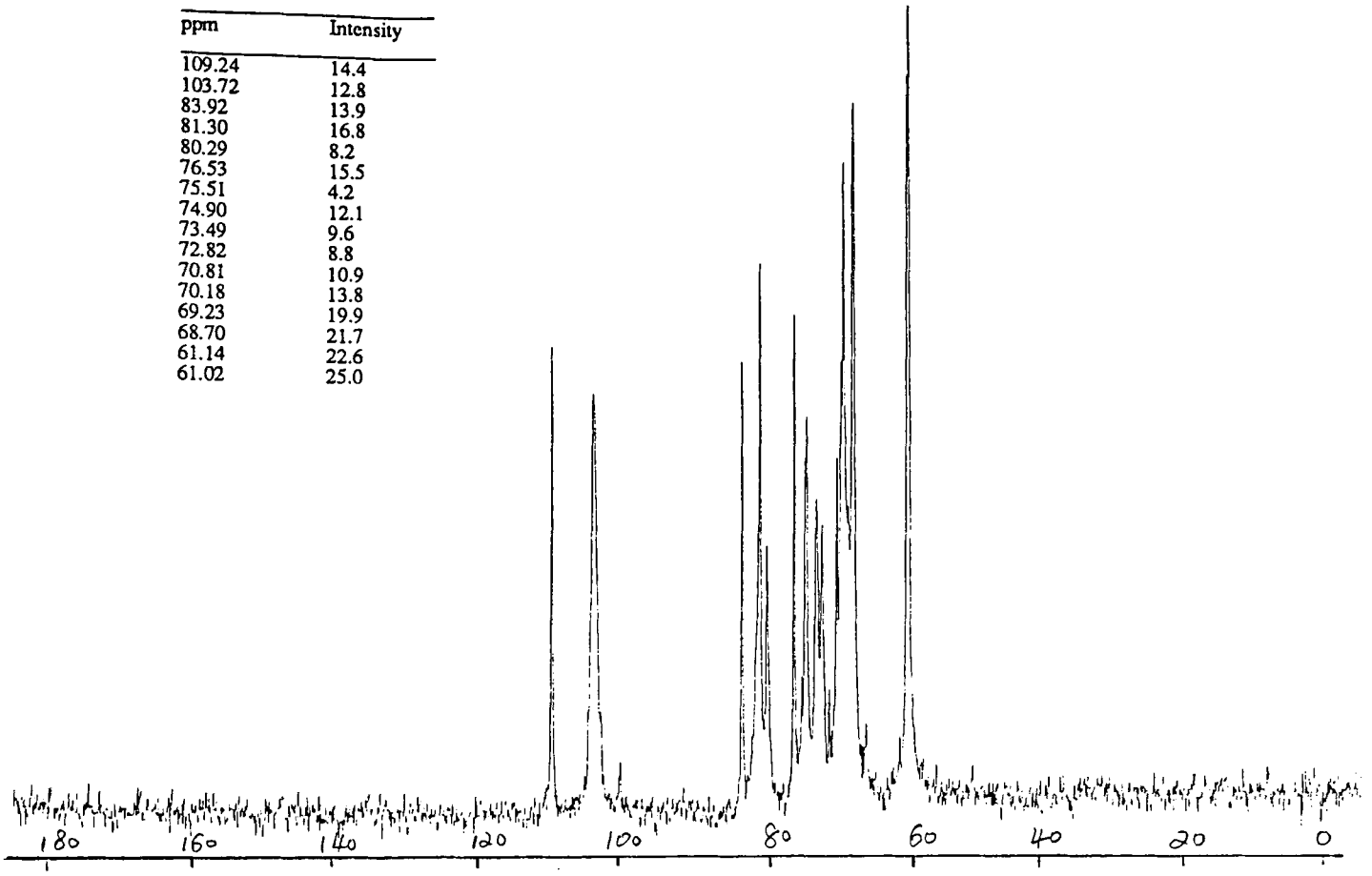

Fig. 3.1 ^{13}C NMR spectrum for *A. senegal* gum after 6 Smith-degradations

ppm	Intensity
109.32	4.2
103.54	11.4
83.78	13.9
82.02	8.2
81.33	6.3
80.30	3.5
76.64	5.7
75.14	15.9
73.56	7.5
72.78	13.4
70.82	15.5
70.20	15.3
69.23	7.8
68.70	22.7
61.13	25.0

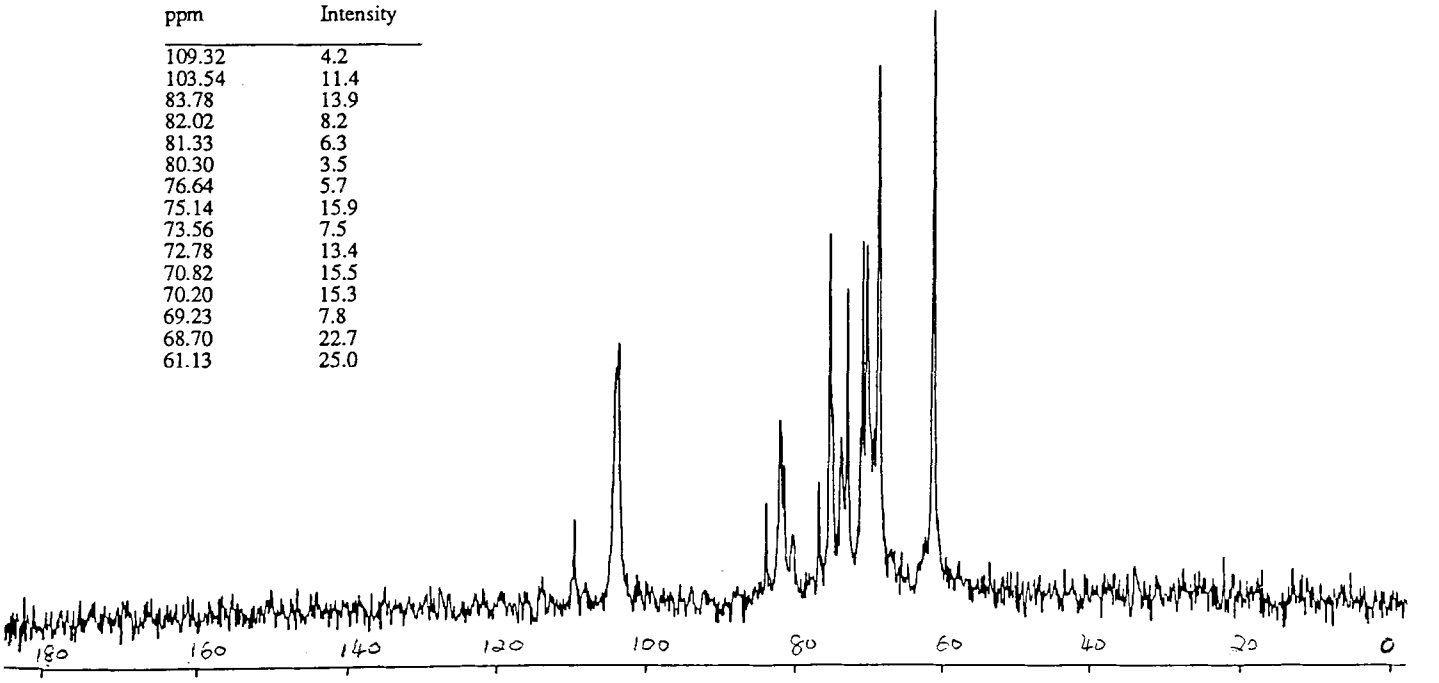

Fig. 3.2 ^{13}C NMR spectrum for *A. senegal* gum after 7 Smith-degradations

Table 3.2 ^{13}C NMR assignments for *Acacia senegal* gum after 6 Smith-degradations

δ(ppm)	Intensity	Carbon
61.0	25.0	α-L-Araf $C_1\rightarrow$, C_5
61.1	22.6	1,3,β-D-Gal, C_6 and 1,β-D-Gal, C_6
68.7	21.7	1,3,β-D-Gal, C_4 and 1,3,6,β-D-Gal, C_4
69.2	19.9	1,6,β-D-Gal, C_4 & C_6 and 1,β-D-Gal, C_4
70.2	13.8	1,3,6,β-D-Gal,C_2 & C_6 and 1,3,β-D-Gal,C_2
70.8	10.9	1,β-D-Gal,C_2 and 1,6,β-D-Gal,C_2
72.8	8.8	1,6,β-D-Gal, C_3 and 1,β-D-Gal, C_3
73.5	9.6	1,6,β-D-Gal, C_5 and 1,3,6,β-D-Gal, C_5
74.9	12.1	1,3,β-D-Gal, C_5 and 1,β-D-Gal, C_5
75.5	4.2	(Araf)→3,β-D-Gal, C_5
76.5	15.5	α-L-Araf $C_1\rightarrow$, C_3
80.3	8.2	α-L-Araf $C_1\rightarrow$, C_2
81.3	16.8	1,3,β-D-Gal, C_3 and 1,3,6,β-D-Gal, C_3
83.9	13.9	α-L-Araf $C_1\rightarrow$, C_4
103.7	12.8	1,3,6, β-D-Gal, C_1 and 1,3, β-D-Gal, C_1 1,6, β-D-Gal, C_1 and 1, β-D-Gal, C_1
109.2	14.4	α-L-Araf $C_1\rightarrow$, C_1

Linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-Araf-(1→	109.2	80.3	76.5	83.9	61.0	
β-D-Gal-(1→	103.7	70.8	72.8	69.2	74.9	61.1
→3,6)β-D-Gal-(1→	103.7	70.2	81.3	68.7	73.5	69.2
→3)β-D-Gal-(1→	103.7	70.2	81.3	68.7	74.9	61.1
(Araf)→3)β-D-Gal-(1→					75.5	
→6)β-D-Gal-(1→	103.7	70.8	72.8	69.2	73.5	69.2

Table 3.3 ^{13}C NMR assignments for *Acacia senegal* gum after 7 Smith-degradations

δ(ppm)	Intensity	Carbon
61.05	25.0	1,β-D-Gal, C_6 and 1,3,β-D-Gal, C_6 1,3,6,β-D-Gal, C_6 and α-L-Araf $C_1\rightarrow$, C_5
68.7	22.7	1,3,β-D-Gal, C_4 and 1,3,6,β-D-Gal, C_4
69.2	7.8	1,6,β-D-Gal, C_4 and C_6 and 1,β-D-Gal, C_4
70.2	15.3	1,3,6,β-D-Gal, C_2 and 1,3,β-D-Gal, C_2
70.8	15.5	1,β-D-Gal, C_2 and 1,6,β-D-Gal, C_2
72.8	13.4	1,β-D-Gal, C_3 and 1,6,β-D-Gal, C_3
73.6	7.5	1,6,β-D-Gal, C_5 and 1,3,6,β-D-Gal, C_5
75.1	15.9	1,3,β-D-Gal, C_5 and 1,β-D-Gal, C_5
76.6	5.7	α-L-Araf $C_1\rightarrow$, C_3
80.3	3.5	α-L-Araf $C_1\rightarrow$, C_2
81.3	6.3	1,3, β-D-Gal, C_3
82.0	8.2	1,3,6,β-D-Gal, C_3
83.8	13.9	α-L-Araf $C_1\rightarrow$, C_4
103.5	11.4	1,3,6,β-D-Gal,C_1 and 1,3,β-D-Gal,C_1 1,6,β-D-Gal,C_1 and 1,β-D-Gal,C_1
109.3	4.2	α-L-Araf $C_1\rightarrow$, C_1

Linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-Araf-(1→	109.3	80.3	76.6	83.8	61.05	
β-D-Gal-(1→	103.5	70.8	72.8	69.2	75.1	61.05
→3,6)β-D-Gal-(1→	103.5	70.2	82.0	68.7	73.6	69.2
→3)β-D-Gal-(1→	103.5	70.2	81.3	68.7	75.1	61.05
→6)β-D-Gal-(1→	103.5	70.8	72.8	69.2	73.6	69.2

Two typical whole gum ^{13}C NMR spectra were chosen to study the relationship between the assignments and the linkages. Table 3.4 lists both the integral and intensity figures for *Acacia maidenii* gum. The spectrum (Fig. 3.3) indicates that β-D-Gal(103.8, 103.1 ppm), β-D-GlupA(102.8, 102.6 ppm) and α-L-Rham(100.6 ppm) are the main sugars plus a small amount of α-L-Arap(104.4 ppm). The peaks at 16.6 and 175.3 ppm give support to this conclusion. Additional overall structural information can also be obtained by an examination of the ^{13}C spectrum line-widths: narrow peaks indicate the terminal groups or side-chains; broader peaks indicate the greater variety of chemical environment involved in the backbone of a polysaccharide (Selvendran and Ryden 1990). The structure of *A. maidenii* gum therefore apparently consists of

Rham1→4,GlupA1→1,6,Gal
Rham1→4,GlupA1→1,3,6,Gal
Arap1→1,3,6 Gal

and the two split peaks (102.8, 102.6 ppm) indicate that there are at least two different environment linkages for GlupA1→. The sharp peak at 79.0 ppm indicates the (Rham1)→4GlupA linkage and peaks from 81.3 to 82.0 ppm indicate that C_3 of Gal is involved in restricted segmental motion in the core arising from different chemical environments.

The line-broadening or splitting of the uronic acid resonances can be caused by interaction with the traces of divalent cations (Selvendran and Ryden 1990) which normally exist in natural gums. An O-CH_3 group always contributes a signal around 55~61 ppm which depends on both the sugar and carbon position linked (Haverkamp et al. 1975); and acetyls will appear around 20~23 ppm. Table 3.4 lists the possible interpretations of all the resonances in the spectrum (Fig.3.3).

The neutral sugar composition of *A. maidenii* gum was found to be Gal:Ara:Rha=57:5:34 by paper chromatography (PC) after acid hydrolysis. The very small proportion of arabinose present in this gum is confirmed by its ^{13}C NMR spectrum (Fig. 3.3). Moreover, the ^{13}C NMR sugar spectrum of acid hydrolysed *A. maidenii* gum (Fig. 3.4) also shows very strong galactose and rhamnose carbon resonances (96.36 and 92.19 ppm for C_1 of β- and α- D-Gal; 93.88 and 93.41 ppm for C_1 of α- and β- L-Rha), and very low intensity for arabinose carbon resonances (96.54 and 92.4 ppm for C_1 of α- and β L-Arap).

Acacia maidenii gum

ppm	Integral	Intensity
175.62	0.4	1.2
175.34	2.2	2.7
104.38	1.1	1.6
103.10	1.2	2.4
102.84	3.3	5.2
102.61	4.3	6.1
101.08	0.4	1.1
100.66	7.7	11.9
82.07	1.9	2.3
81.70	3.0	2.8
81.35	1.9	2.3
79.88	0.9	1.2
79.04	9.3	10.5
76.16	10.8	8.5
75.18	2.5	1.9
74.26	9.8	10.3
73.59	6.6	7.3
72.63	4.4	5.0
72.22	2.1	3.9
71.96	9.4	12.2
70.70	8.1	6.9
70.32	10.9	15.0
70.07	14.8	16.8
68.93	18.0	16.2
68.61	5.7	6.5
67.83	1.1	1.5
67.54	0.8	1.2
61.11	0.8	1.3
16.56	8.4	10.4

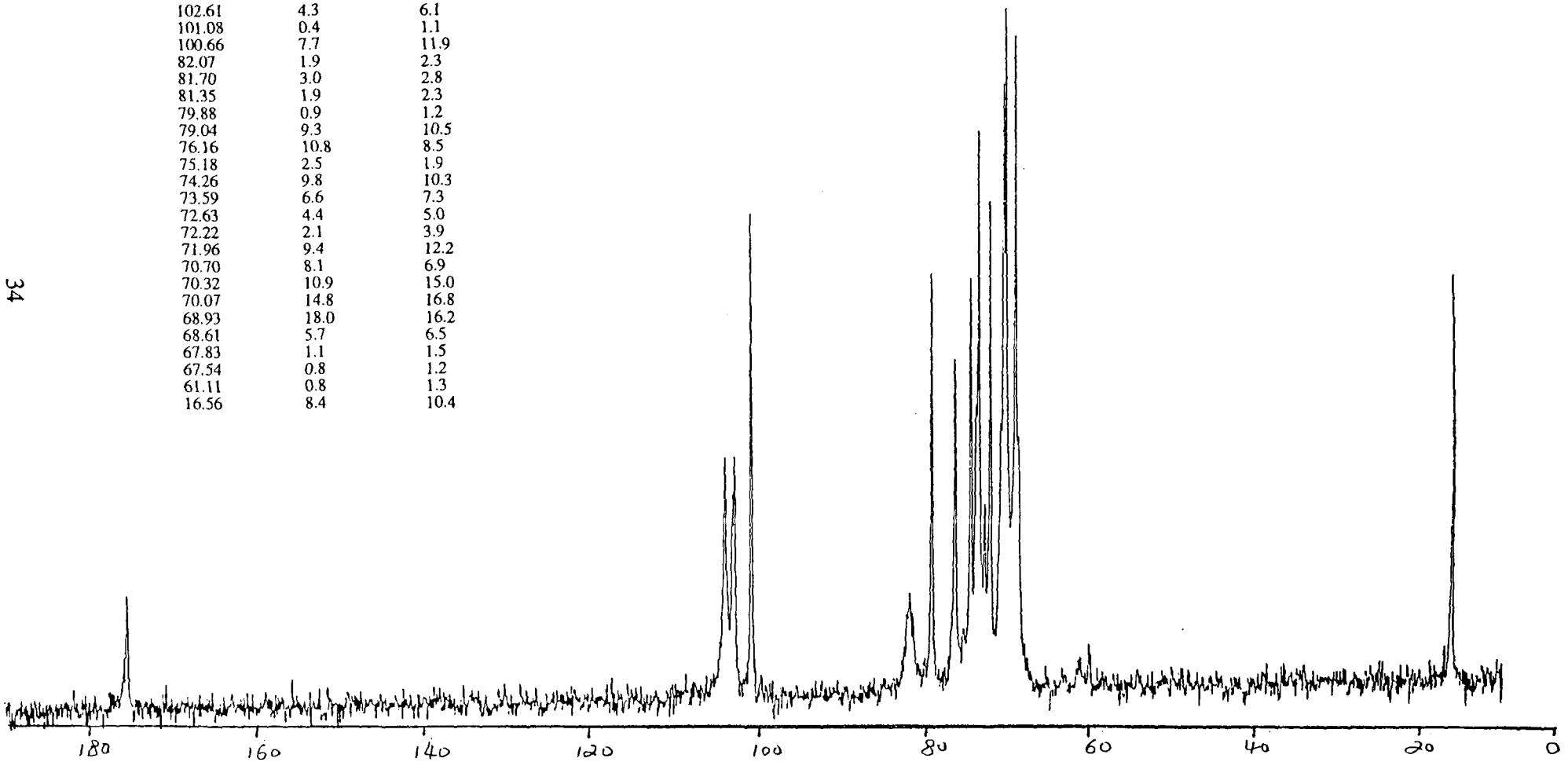

Fig. 3.3 ^{13}C NMR spectrum for *Acacia maidenii* gum

Assignments of sugars in *A. maidenii* gum after acidic hydrolysis

δ(ppm)	Intensity	Carbon
16.75	13.0	Rham 6
60.92	17.3	β-Gal 6
61.12	8.9	α-Gal 6
62.42	1.8	β-Arap 5
68.20	14.2	α-Rham 5
68.27	12.6	α-Gal 3
68.67	19.2	β-Gal 4
69.09	12.6	α-Gal 4
69.23	11.8	α-Gal 2
69.87	12.7	α-GlupA 4,α-Rham 3
70.38	9.5	α-Gal 5
70.73	11.9	β-GlupA 4,α-GlupA 5,α-Rham 2
71.26	8.6	β-Rham 2
71.74	24.4	β-Rham 5
71.79	25.0	β-Rham 4,β-Gal 2
72.10	13.8	α-Rham 4,α-GlupA 2
72.49	7.6	β-Rham 3
72.70	21.7	β-Gal 3
73.64	2.5	β-GlupA 2
73.89	5.3	β-GlupA 3,5
75.05	19.1	β-Gal 5
92.02	4.3	α-GlupA 1
92.19	9.7	α-Gal 1
93.41	7.1	β-Rham 1
93.88	12.7	α-Rham 1
95.78	5.5	β-GlupA 1
96.36	19.9	β-Gal 1
96.54	2.5	α-Arap 1
102.28	3.2	GlupA $C_1\rightarrow$
175.89	2.1	β-GlupA 6
176.85	1.6	α-GlupA 6

Neutral sugar ratios obtained by PC mehtod:
Gal:Ara:Rha=57:5:34

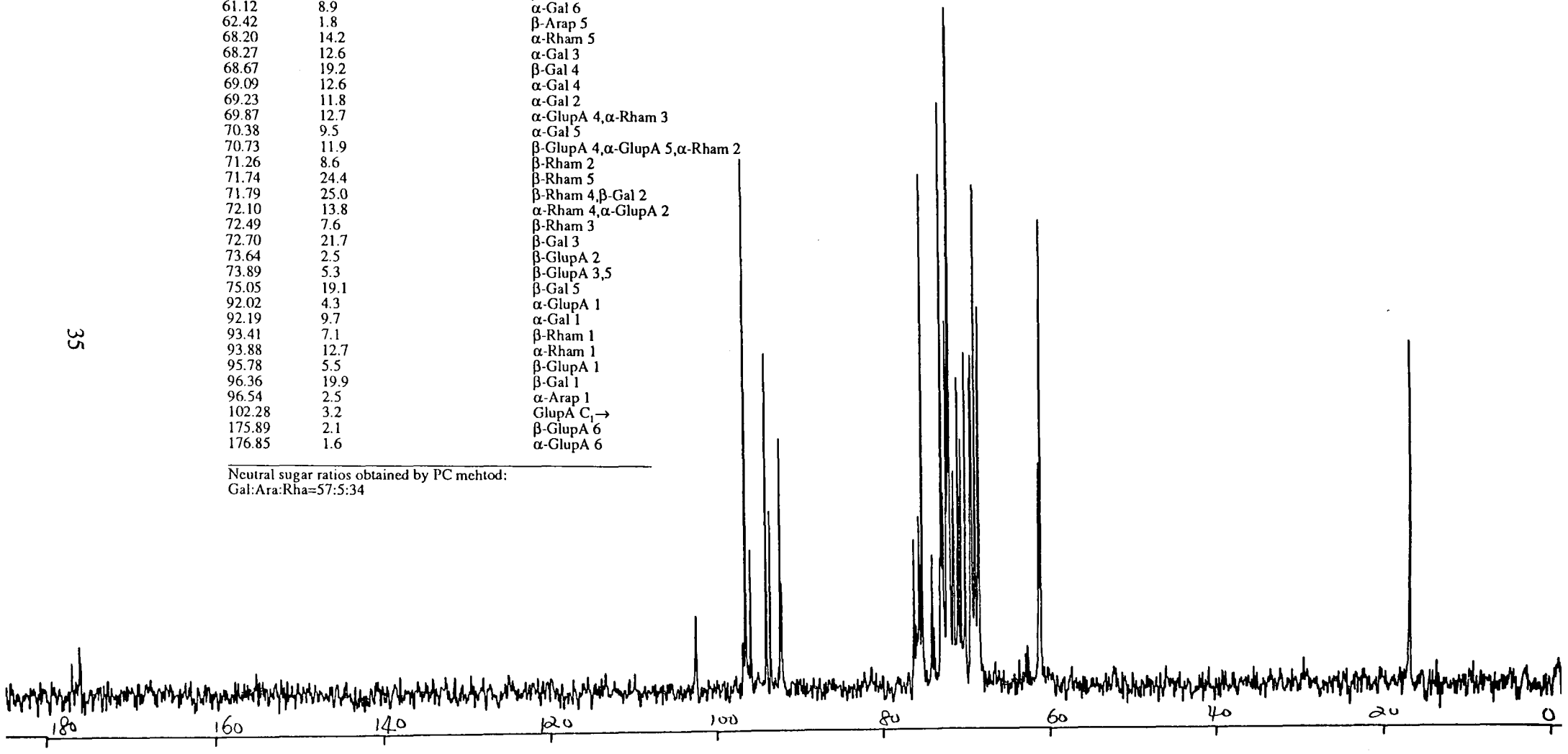

Fig. 3.4 ^{13}C NMR spectrum for *A. maidenii* gum after acidic hydrolysis

Table 3.4 ^{13}C NMR assignments for *Acacia maidenii* whole gum

δ(ppm)	integral	Intensity	Carbon
16.6	8.5	10.4	α-L-Rham, C_6
67.8	1.1	1.5	α-L-Arap $C_1\rightarrow$, C_5
68.6	5.7	6.5	α-L-Rham, C_5
			1,3,β-D-Gal,C_4
68.9	18.0	16.2	1,3,6,β-D-Gal, C_4 or C_6
			1,6,β-D-Gal, C_4 or C_6
			α-L-Arap $C_1\rightarrow$, C_4
70.1	14.8	16.8	α-L-Rham, C_3
			1,3,6, & 1,3,4,6,β-D-Gal, C_6
70.3	10.9	15.0	1,3 & 1,3,6,& 1,3,4,6,β-D-Gal,C_2
70.7	8.1	6.9	1,6,β-D-Gal,C_2
			α-L-Rham, C_2
72.0	9.5	12.2	α-L-Rham, C_4
			α-L-Arap $C_1\rightarrow$, C_2
72.2	2.1	3.9	α-L-Arap $C_1\rightarrow$, C_3
72.6	4.4	5.0	1,6,β-D-Gal,C_3
73.3	12.3	14.0	1,(4),β-D-GlupA, C_2
			1,3,4,6,β-D-Gal, C_5
73.6	6.6	7.3	1,6, & 1,3,6,β-D-Gal,C_5
74.3	9.8	10.3	1,(4), β-D-GlupA, C_3
			1,3,4,6,β-D-Gal, C_4
75.2	2.5	1.9	1,3,β-D-Gal,C_5
76.2	10.8	8.5	1,(4), β-D-GlupA, C_5
79.0	9.3	10.4	1,4,β-D-GlupA, C_4
81.3	1.9	2.3	1,3,& 1,3,6,β-D-Gal,C_3
81.7	3.0	2.8	1,3,4,6,β-D-Gal,C_3
82.0	1.9	2.3	1,3,6,β-D-Gal,C_3
100.6	7.7	11.9	α-L-Rham, C_1
102.6	4.3	6.1	1,(4),β-D-GlupA,C_1
102.8	3.3	5.2	1,(4),β-D-GlupA,C_1
103.1	1.2	2.4	1,3, & 1,6,β-D-Gal,C_1
103.8	9.2	6.1	1,3,(4),6,β-D-Gal,C_1
104.4	1.1	1.6	α-L-Arap $C_1\rightarrow$,C_1
175.3	2.2	2.7	1,(4),β-D-GlupA, C_6

Linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-Rham-(1→	100.6	70.7	70.1	72.0	68.6	16.6
→4)β-D-GlupA-(1→	102.6 102.8	73.3	74.3	79.0	76.2	175.3
α-L-Arap-(1→	104.4	72.0	72.2	68.9	67.8	
→3,6)β-D-Gal-(1→	103.8	70.3	81.3 82.0	68.9	73.6	70.1 68.9
→3,4,6)β-D-Gal-(1→	103.8	70.3	81.7	74.3	73.3	70.1 68.9
→6)β-D-Gal-(1→	103.1	70.7	72.6	68.9	73.6	68.9
→3)β-D-Gal-(1→	103.1	70.3	81.3	68.6	75.2	61.1

Fig. 3.5 shows the spectrum for an *Acacia* spp. gum received from Kenya in 1990. It indicates the presence of four sugars e.g. β-D-Gal, β-D-GlupA, α-L-Araf and α-L-Rham. A careful examination of the anomeric regions shows at least two types of terminal α-L-Araf, 1,3,α-L-Araf (because no signals between 85~90 ppm indicate that only C_3 or C_5 linkages exist), at least two locations for both β-D-Gal and 1,4,β-D-GlupA, and terminal α-L-Rham.

Sugar units

(109.4) 1,α-L-Araf type one
(109.2) 1,α-L-Araf type two
(108.3) 1,3,α-L-Araf
(103.6) 1,3,6,β-D-Gal
(103.3) 1,3,β-D-Gal
(102.9) 1,4,β-D-GlupA type one
(102.6) 1,4,β-D-GlupA type two
(100.6) 1,α-L-Rham

By paper chromatography, the sugars present in this *Acacia* gum after acid hydrolysis were found to be galactose, arabinose, rhamnose and glucuronic acid in the ratio 44:30:12:15. This agrees with the conclusions made directly from the ^{13}C NMR spectrum (Fig. 3.5). Table 3.5 lists the possible interpretations of the resonances in the spectrum of this gum (Fig. 3.5).

Since ^{13}C NMR spectroscopy provides a unique "fingerprint" for each gums, it is a very powerful method for investigating those gums whose chemical analytical data are very similar but which have different functional properties and hence differences in fine structure. Models of polysaccharide structures can be deduced from ^{13}C NMR spectra (both sugar and whole gum spectra) data and can be supplemented from methylation analysis, sequential Smith-degradations and fractional separations. It has been reported that the amino acids which are always linked with polysaccharides through specific sugars can be determined by this method (Breitmaier and Voelter 1978).

*Acacia ssp.*gum

ppm	Integral	Intensity
175.12	5.8	4.8
109.43	12.3	7.8
109.23	14.0	10.4
108.34	7.9	4.9
103.67	38.3	11.3
103.35	12.5	8.6
102.93	8.3	6.6
102.65	8.8	5.6
100.62	13.6	9.2
83.83	26.8	13.5
83.58	12.6	9.2
81.34	43.5	20.2
80.42	10.2	4.3
80.15	14.7	5.8
79.02	14.2	9.0
76.65	30.7	13.5
76.02	14.8	7.3
75.78	11.5	8.4
74.16	28.4	13.4
73.68	22.4	11.7
73.43	17.3	12.3
72.63	24.3	10.5
71.89	21.4	10.7
70.68	27.9	12.0
70.34	31.8	18.0
69.99	24.8	18.6
69.84	29.8	14.2
69.53	26.7	9.9
68.90	24.9	16.4
68.60	42.8	12.6
61.21	46.1	16.9
16.52	13.6	6.9

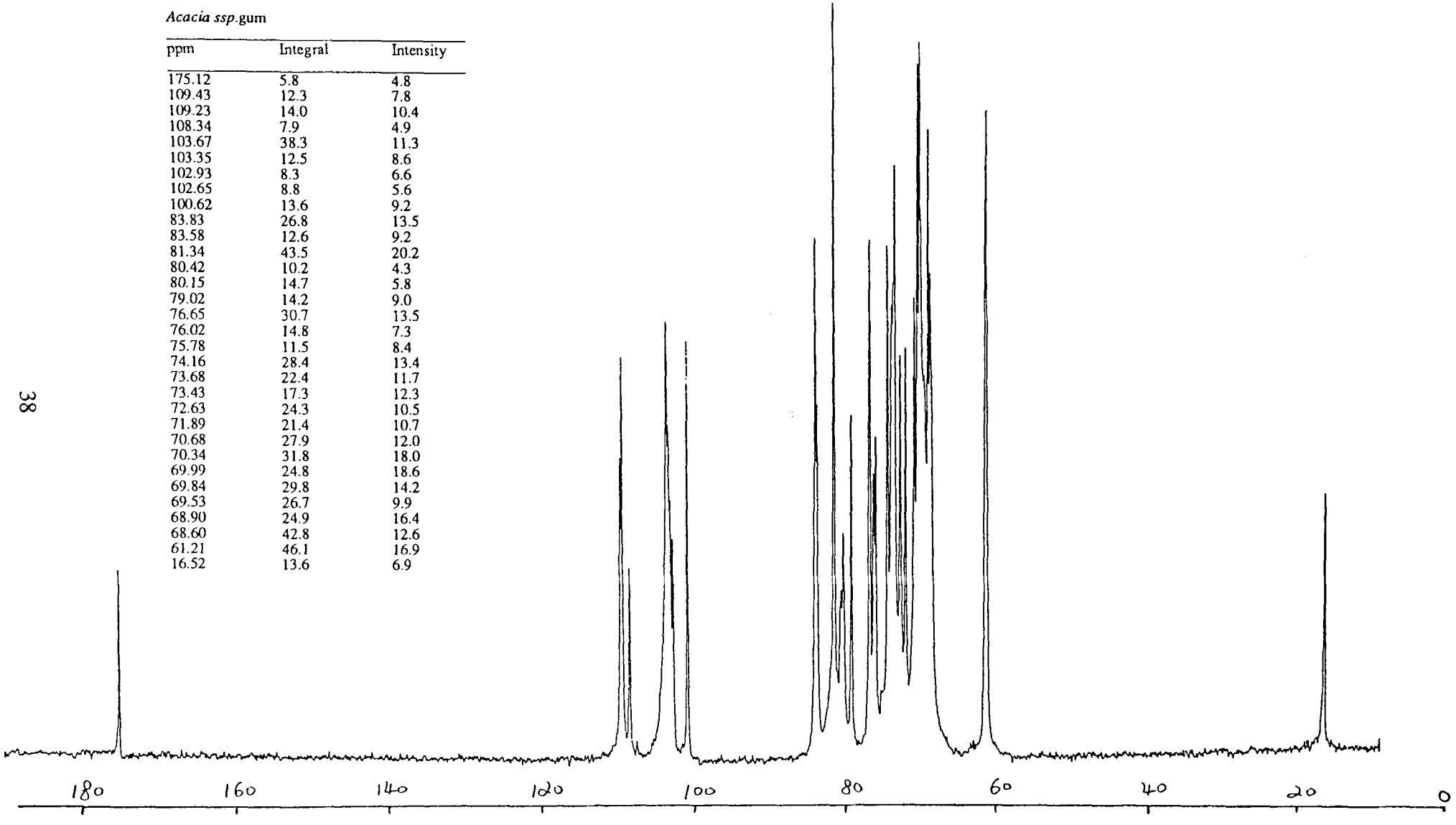

Fig. 3.5 ^{13}C NMR spectrum for *Acacia ssp.* gum from Kenya

Table 3.5 ^{13}C NMR assignments of an *Acacia* spp. gum from Kenya

δ(ppm)	integral	Intensity	Carbon
16.5	13.6	6.9	α-L-Rham, C_6
61.2	46.1	16.9	1, & 1,3,β-D-Gal, C_6 and α-L-Araf $C_1\rightarrow$, C_5
68.6	42.8	12.6	1,3, & 1,6,β-D-Gal, C_4
			α-L-Rham, C_5 and 1,5,α-L-Araf, C_5
68.9	24.9	16.4	1, & 1,3,6,β-D-Gal, C_4 and α-L-Rham, C_2
69.5	26.7	9.9	1,6,β-D-Gal, C_6
69.8	29.8	14.2	1,3,6,β-D-Gal, C_6
70.0	24.8	18.6	1,3,4,6,β-D-Gal, C_6 and α-L-Rham, C_3
70.3	31.8	18.0	1,3, & 1,3,6, & 1,3,4,6,β-D-Gal, C_2
70.7	27.9	12.0	α-L-Rham, C_2
			1, & 1,6,β-D-Gal, C_2
71.9	21.4	10.7	α-L-Rham, C_4
72.6	24.3	10.5	1, & 1,6,β-D-Gal, C_3
73.4	17.3	12.3	1,(4),β-D-GlupA, C_2
			1,3,4,6,β-D-Gal, C_5
73.7	22.4	11.7	1,6,& 1,3,6,β-D-Gal, C_5
74.2	28.4	13.4	1,(4),β-D-GlupA, C_3
			1,3,4,6,β-D-Gal, C_4
75.8	11.5	8.4	1,3,β-D-Gal, C_5?
76.0	14.8	7.3	1,(4),β-D-GlupA, C_5
76.6	30.7	13.5	1,(5),α-L-Araf, C_3
79.0	14.2	9.0	1,4,β-D-GlupA, C_4
80.1	14.7	5.8	α-L-Araf $C_1\rightarrow$, C_2
80.4	10.2	4.3	1,5, α-L-Araf, C_2
81.3	43.5	20.2	1,3,& 1,3,6,&1,3,4,6,β-D-Gal, C_3
83.6	12.6	9.2	1,5,α-L-Araf, C_4
83.8	26.8	13.5	α-L-Araf $C_1\rightarrow$, C_4
100.6	13.6	10.9	α-L-Rham, C_1
102.6	8.8	5.6	1,(4),β-D-GlupA, C_1
102.9	8.3	6.6	1,(4),β-D-GlupA, C_1
103.3	12.5	8.6	1,& 1,3,&1,6,β-D-Gal, C_1
103.6	38.3	11.3	1,3,6,& 1,3,4,6,β-D-Gal,C_1
108.3	7.9	4.9	1,5,α-L-Araf, C_1
109.2	14.0	10.4	α-L-Araf $C_1\rightarrow$, C_1
109.4	12.3	7.8	α-L-Araf $C_1\rightarrow$, C_1
175.1	5.8	4.8	1,(4),β-D-GlupA, C_6

Linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-Rham-(1→	100.6	70.7	70.0	71.9	68.6	16.5
→4)β-D-GlupA-(1→	102.6	73.4	74.2	79.0	76.0	175.1
	102.9					
α-L-Araf-(1→	109.4	80.1	76.6	83.8	61.2	
	109.2					
→5)α-L-Araf-(1→	108.3	80.4	76.6	83.6	68.6?	
→3,6)β-D-Gal-(1→	103.6	70.3	81.3	68.9	74.2	69.8
→3,4,6)β-D-Gal-(1→	103.6	70.3	81.3	74.2	73.4	70.0
→6)β-D-Gal-(1→	103.3	70.7	72.6	68.6	73.7	69.5
→3)β-D-Gal-(1→	103.3	70.3	81.3	68.6	75.8?	61.2
β-D-Gal-(1→	103.3	70.7	72.6	68.9	(75)	61.2

Chapter 4
Studies of Gum Arabic (Acacia senegal (L.) Willd.)

4.1 Introduction

Gum arabic is probably the oldest and best known of the plant exudates. It is reported to have been first used in the USA in 1880; but it has been an article of commerce for several thousands of years and its earliest uses are lost in antiquity. In 1961, gum arabic was classified by the US Food and Drug Administration as being Generally Recognized As Safe (GRAS) as a food stabiliser and in 1974 it was re-affirmed as GRAS for use in foodstuffs.

Gum arabic is defined as the "dried gummy exudate obtained from stems or branches of *Acacia senegal* (L.) Willd. or the closely related species of Acacia"(EEC 1978), and, as such, it is a permitted foodstuffs additive (E414) within the EEC. The definition adopted by the Joint FAO/WHO Committee on Food Additives (JECFA) is essentially similar (FAO 1990). Taxonomically, only *Acacia laeta, A. mellifera, A. polyacantha*, and comparatively rare species within the "*Acacia senegal* complex" (Rose 1979; Brenan 1983) are admissible as closely related species of *Acacia senegal*, but none of these are used to any extent to produce gum for commercial purposes. The most recent specification for gum arabic requires (FAO 1990) that the specific rotation must be between -26° to -34° with a nitrogen content of 0.27 to 0.39%.

In the market, virtually all true gum arabic comes from the tree *Acacia senegal* (L.) Willd. and the largest producer is the Republic of Sudan (about 85% of the world total production). But a small quantity is also available in other countries, such as Uganda; Niger; Kenya; Chad; Senegal; Ethiopia; Tanzania etc.. Gum production (tapping) is stimulated by stripping the bark from upper branches, then the gum is collected by hand three weeks later.

Natural gum arabic occurs as yellowish-white spheroidal nodules of varying size or as angular fragments and is sometimes mixed with darker fragments. It consists mainly of high molecular-weight polysaccharides and their calcium, magnesium and potassium salts and other cations (eg. iron, sodium, etc.). Complete hydrolysis with dilute acid yields D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. In addition to sugars, it has been found that a small amount of protein is always one of the components of gum arabic (Anderson et al. 1972). Therefore, gum arabic could

be described as an acidic proteinaceous polysaccharide or a kind of arabinogalactan-protein (Akiyama 1984). Within the polysaccharide field gum arabic is unique in having high solubility, forming aqueous solutions up to concentrations of 50% (w/v).

Commercially, good quality gum arabic is used in the pharmaceutical, cosmetic and food industries, whereas inferior grades are used in lithography, paints and inks, foundry sands and ceramics etc.. The main use of gum arabic is, however, in the food industry, to influence the viscosity, body and texture of foods and to impart certain properties to food that cannot be obtained from other materials; eg. as an adhesive in glazes and toppings for bakery products, as a foam stabiliser in beer, as a sugar crystallisation inhibitor in syrups and candies, and as a stabiliser in many flavour emulsions. Because it is odourless, colourless, tasteless and completely water-soluble it does not affect the flavour, colour and odour of other food ingredients. Gum arabic is assessed toxicologically as a safe foodstuffs additive to which the "not specified" category of Acceptable Daily Intake (ADI) was assigned in 1982 (FAO/WHO 1982).

Gum arabic also has applications in pharmaceutical products and cosmetics as a stabilizing and emulsifying aid, as a suspending agent, as a demulcent in cough syrups, as an adhesive and binder in tablets and as an emulsion stabiliser in protective creams and lotions etc..

Thus, it is very important to have precise specifications for the purity and identity of gum arabic for trade and enforcement purposes as there are no toxicological data of any kind for any *Acacia* gum other than that from *Acacia senegal.*

4.2 Structural Studies by Carbon-13 NMR Method

4.2.1 Introduction

Although different structural models for gum arabic exuded by *Acacia senegal* have been proposed based on the different evidence obtained, a definite structure for this highly branched polysaccharide has not yet been elucidated. Structural investigations by methylation and acidic degradations and by sequential Smith-degradation (Goldstein et al. 1965; Anderson et al. 1966b) have shown that the gum arabic molecule consists of a highly branched galactose core with side chains containing arabinose, rhamnose and glucuronic acid. A small proportion of proteinaceous material (2-3%) is now known to be an integral part of the structure,

with some amino acids in peripheral positions and proportionally more, particularly Hyp, associated with the galactan core (Anderson and McDougal 1987a).

By using a modelling approach, Street and Anderson proposed a structure (Street and Anderson 1983) which showed the possible linkages between the galactose backbone and the sugars in the side-chains. Churms also showed the structure to possess a high degree of regularity and proposed that the molecules consisted of 64-subunits; each of molecular mass 8000, which may be arranged linearly or may be randomly disposed (Churms et al. 1983). It was concluded that gum arabic consists of a $\beta 1 \rightarrow 3$ linked galactopyranose backbone with branches containing $\beta 1 \rightarrow 6$ links linked in turn with galactopyranose, arabinopyranose, arabinofuranose, rhamnopyranose, glucuronic acid and 4-O-methylglucuronic acid end-groups. The proteinaceous material, which is important for the emulsifying functional properties (Dickinson et al. 1991) was not taken into account in those structural investigations. In 1987, Connolly et al. described the structure of gum arabic in terms of the "Wattle Blossom Model" which includes the proteinaceous component and suggested that a number of arabinogalactan blocks of molecular mass $\sim 2 \times 10^5$ are linked to a polypeptide chain (Connolly et al. 1987). Recently, a "Gum Arabic Glycoprotein Model" has been described by Lamport et al. who suggested that "Gum Arabic Glycoprotein is a Twisted Hairy Rope"; the polysaccharide substituent is considered to have an average of 30 sugar residues on 10 to 12 amino acid residues which is the basic repetitive unit of the polypeptide backbone. It contains a semiflexible (Hyp/Pro-rich) polypeptide backbone of >400 residues, and it is rodlike based on electron microscopy (Wu et al. 1991).

All those postulations depend on the observations from different experimental techniques and most of them involve fractionation studies. The ^{13}C NMR spectroscopy method can provide gum arabic's natural structural "fingerprint" non-destructively. The information obtained by this method includes the relative proportions of the constituent sugars; the configuration of the interglycosidic linkages and linkage types, with an indication of the mobility of the residues.

4.2.2 Results and Discussion

Fig. 4.1 is a ^{13}C NMR spectrum for a sample of *Acacia senegal* gum , which was obtained from Sudan in 1990 and dissolved in D_2O then run overnight. It shows gum arabic's structural molecular fingerprint obtained by ^{13}C NMR spectroscopy. The sugars present in the whole gum can be deduced from the chemical shifts of the

Acacia senegal gum

ppm	Integral	Intensity	ppm	Integral	Intensity
175.00	5.0	4.0	76.71	16.3	5.8
109.45	16.9	5.5	76.31	1.6	3.9
108.08	1.7	3.8	76.01	24.4	8.4
103.52	15.8	4.5	74.80	5.4	3.1
102.87	15.7	7.1	74.74	1.1	3.1
102.53	5.6	3.7	74.59	1.7	3.2
100.60	15.6	10.7	74.15	22.1	10.7
100.00	11.1	6.9	73.23	46.5	13.4
99.12	3.4	2.1	72.37	2.3	3.3
84.85	8.6	3.4	71.86	20.2	11.4
84.45	1.2	2.0	71.26	12.5	9.2
83.90	14.5	5.4	70.94	1.8	5.2
83.51	5.6	3.8	70.22	35.1	15.1
83.21	1.4	2.7	69.98	33.6	17.6
82.75	13.7	5.7	69.17	33.7	19.2
82.36	1.4	2.4	68.86	24.6	17.8
81.97	2.6	3.1	68.53	5.4	8.9
81.32	28.7	8.6	68.26	26.4	11.1
80.61	1.9	3.5	63.21	2.7	1.7
80.11	16.9	5.9	61.19	56.6	25.0
79.69	3.6	4.6	60.36	1.1	1.2
79.60	16.3	8.8	59.92	1.4	1.4
78.53	1.0	1.4	16.49	10.3	10.3

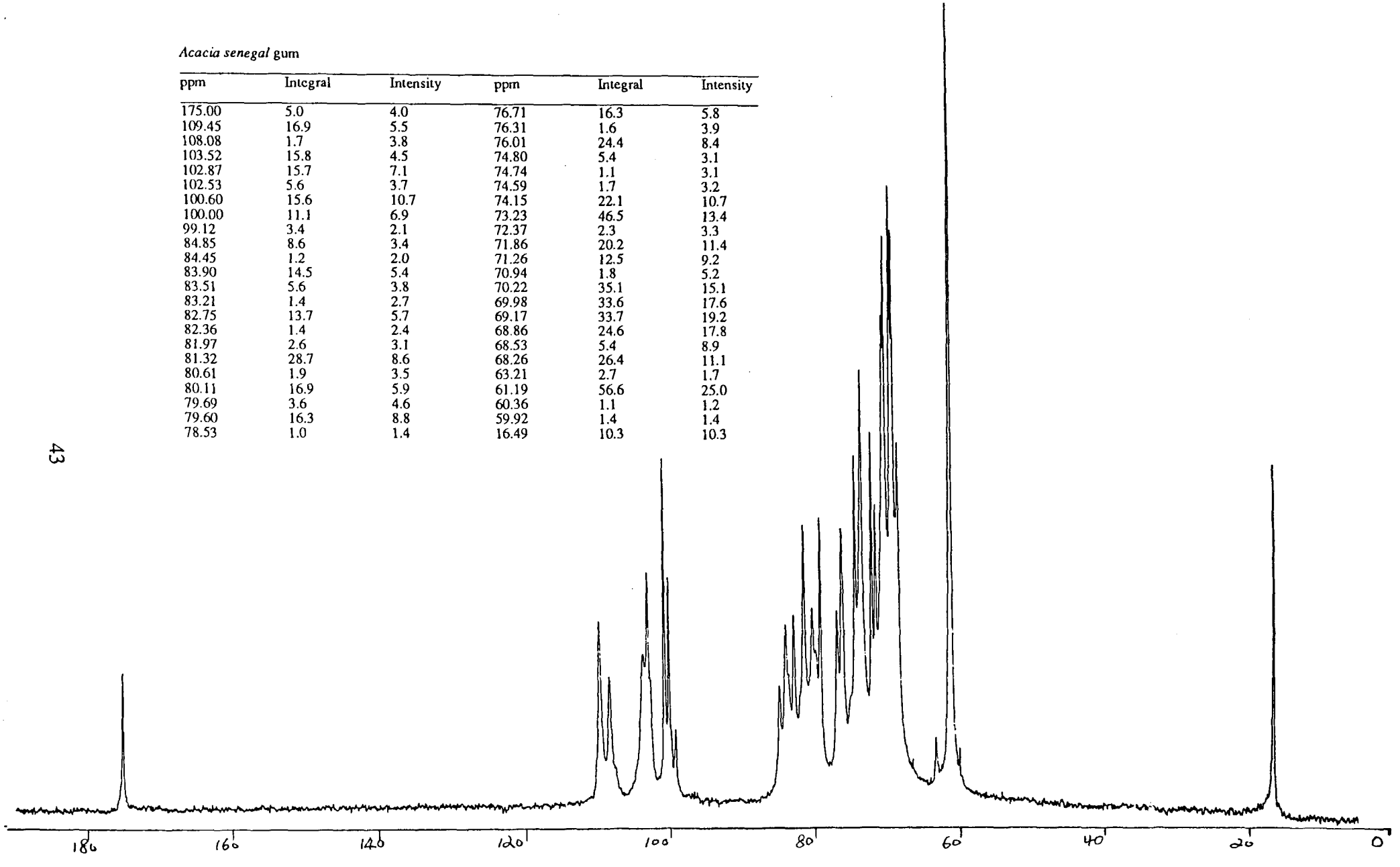

Fig. 4.1 ^{13}C NMR spectrum for *A. senegal*(L.) Willd. gum from Sudan

anomeric resonances by comparing with Tables 3.1(a) and (b). A small amount of β-L-Arap1→ was detected in this way; although it was reported in earlier structural studies (Anderson and Dea 1968), it was not pointed out in a ^{13}C NMR study on gum arabic (Defaye and Wong 1986).

Table 4.1(a) The structural composition of *A. senegal* (L.)Willd. gum from Sudan, as indicated by the anomeric sugar assignments of the ^{13}C NMR spectrum

Chemical shift (ppm)	C_1 of
109.4	α-L-Araf(1→
108.1	→α-L-Araf(1→
103.6	→β-D-Gal(1→
102.9	→β-D-Gal(1→ and β-D-Gal(1→
102.6	→β-D-GlupA(1→
100.6	α-L-Rham(1→
100.0	α-D-Gal→ and →α-D-Gal(1→
99.2	β-L-Arap(1→

Table 4.1(a) lists only the anomeric peak assignments corresponding with C_1 of different sugar rings. Because of the different environmental linkages in the overall structure, the chemical shifts at 109.4 and 108.1 ppm indicate that at least two types of α-L-Araf exist; one is in a terminal position and the other type may be in internal positions respectively. Differently linked β-D-galactose residues are also present (shifts at 102.9 and 103.6 ppm). The full assignments interpreted are listed in Table 4.1(c). The carbon in the -OCH_3 group in 4-O-Me-β-D-GlupA contributes the 59.9 ppm signal (Matwiyott et al. 1976; Dorman and Roberts 1970; Gorin and Mazurek 1975). The chemical shift of aldofuranose residues is at 106-109 ppm (Casu 1985), and resonances at 99.2-100 ppm and 108.1-109.4 ppm represent β-L-arabinopyranoside and α-L-arabinofuranoside residues (Reuben 1984). Table 4.1(b) shows the corresponding assignments for all carbons. The percentage of each neutral monosaccharide constituent present calculated from the integral of Fig. 4.1 shows internal α-L-Araf (19%), terminal α-L-Araf (13%), β-L-Arap (4%), β-D-Gal (35%), α-D-Gal (12%), α-L-Rha (17%); the ratio of Gal:Ara:Rha=47:36:17 is close to the results obtained by chemical analysis (46:38:16).

The whole gum structure consists of α-L-Rham and β-L-Arap as terminal groups; β or α-D-GlupA is linked 1→4 to galactose. The core branched framework contains a complicated range of linkages; 1,3,4,6; 1,3,4; 1,3,6; 1,3 and 1,4 linkages are all present. The α-L-Araf internal linkage always involves the 3 or 5 positions because

there is no assignment between 85 and 90 ppm in the spectrum, indicating that the C_2 position is not linked to another sugar ring. For *A. senegal* gum the maximum resonance is always at 61.2 ppm which represents both the β-Gal C_6 and the α-Araf C_5 groups. A small peak at 63.2 ppm shows β-Arap C_5. Previous methylation analysis has shown all those types of linkage to exist in the gum (Joseleau and Ullmann 1990; Street and Anderson 1983; Churms et al. 1983).

The ^{13}C NMR spectrum pattern of gum arabic can be used as a unique, unambiguous, ultimate referee method to check the identity of commercial samples of gum which may not be permitted in food because of commercial blending practices involving other gums that are not included in approved lists.

Table 4.1(b) ^{13}C NMR chemical shifts(ppm) for *A. senegal* gum and their assignments to the various monosaccharide constituents

Linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-Rham(1→	100.6	70.2	70.0	71.9	68.3	16.5
→4)β-D-GlupA(1→	102.6	73.3	74.2	79.0	76.0	175.1
α-D-Gal(1→	100.0	68.5	68.9	70.0	71.3	61.2
β-D-Gal(1→	102.9	70.2	73.3	68.3	74.8	61.2
				68.9	76.0	
→3)β-D-Gal(1→	102.9	70.2	81.3	68.3	74.8	61.2
→6)β-D-Gal(1→	102.9	70.7	72.4	68.3	73.3	69.2
→3,6)β-D-Gal(1→	103.6	70.2	81.3	68.9	73.3	70.0
→3,4,6)β-D-Gal(1→	103.6	70.2	81.3	74.2	73.3	69.2
→3,4)β-D-Gal(1→	103.6	70.2	82.0	74.6	73.3	61.2
α-L-Araf(1→	109.4	80.1	76.7	83.8	61.2	
→3)α-L-Araf(1→	108.3	80.1	84.8	83.6	61.2	
(→?,3)α-L-Araf(1→		80.6	84.4			
(→?)α-L-Araf(1→		79.6				
(→?)α-L-Araf(1→		79.7				
β-L-Arap(1→	99.2	70.7	71.9	71.9	63.2	

Table 4.1(c) ^{13}C NMR assignments for the structural features of *A. senegal* gum

δ(ppm)	Integral	Intensity	Carbon
16.5	10.3	10.3	α-L-Rham, C_6
59.9	1.4	1.4	-OCH_3 in 4-O-Me-β-D-GlupA-(1→
61.2	56.6	25.0	1,& 1,3,& 1,3,4,β-D-Gal, C_6
			1,& 1,3,α-L-Araf, C_5 and α-D-Gal, C_6
63.2	2.7	1.7	β-L-Arap C_1→, C_5
68.3	26.4	11.1	1,& 1,3,&1,3,6,β-D-Gal, C_4
			α-L-Rham, C_5
68.5	5.4	8.9	α-D-Gal, C_2
68.9	24.6	17.8	1,& 1,3,6,β-D-Gal, C_4
			α-D-Gal, C_3
69.2	33.7	19.2	1,6,& 1,3,4,6,β-D-Gal, C_6
70.0	33.6	17.6	1,3,6,β-D-Gal, C_6
			α-L-Rham, C_3 and α-D-Gal, C_4
70.2	35.1	15.1	1,& 1,3,& 1,3,4,& 1,3,6,&1,3,4,6,β-D-Gal, C_2
			α-L-Rham, C_2
70.7	1.8	5.2	1,6,β-D-Gal, C_2 and β-L-Arap C_1→, C_2
71.3	18.5	9.2	α-D-Gal, C_5
71.9	20.2	11.4	α-L-Rham,C_4
			β-L-Arap C_1→, C_3 & C_4
72.4	2.3	3.3	1,6,β-D-Gal, C_3
73.3	46.5	13.4	1,β-D-Gal, C_3
			1,6,& 1,3,4,& 1,3,6,& 1,3,4,6,β-D-gal, C_5
			1,(4),β-D-GlupA, C_2
74.2	22.1	10.7	1,(4),β-D-GlupA, C_3 and 1,3,4,6,β-D-Gal, C_4
74.6	1.7	3.2	1,3,4,β-D-Gal, C_4
74.8	5.4	3.1	1,3,β-D-Gal, C_5
76.0	24.4	8.4	1,(4),β-D-GlupA, C_5 and 1,β-D-Gal, C_5
76.7	16.3	5.8	α-L-Araf C_1→, C_3
79.0	16.3	8.8	1,4,β-D-GlupA, C_4
79.6	5.9	4.3	α-L-Araf, C_2
79.7	3.6	4.6	α-L-Araf, C_2
80.1	16.9	5.9	1,(3),α-L-Araf, C_2
80.6	1.9	3.5	α-L-Araf, C_2
81.3	28.7	8.6	1,3,& 1,3,6, & 1,3,4,6,β-D-Gal, C_3
82.0	2.6	3.1	1,3,4,β-D-Gal, C_3
82.8	13.7	5.7	1,3,α-L-Araf, C_4
83.6	5.6	3.8	α-L-Araf, C_4
83.8	14.5	5.4	α-L-Araf C_1→, C_4
84.4	1.2	2.0	1,3, α-L-Araf, C_3
84.8	8.6	3.4	1,3, α-L-Araf, C_3
99.2	3.4	2.1	β-L-Arap C_1→, C_1
100.0	11.1	6.9	α-D-Gal, C_1
100.6	15.6	10.7	α-L-Rham, C_1
102.6	5.6	3.7	1,(4),β-D-GlupA, C_1
102.9	15.7	7.1	1,& 1,3,& 1,6,β-D-Gal, C_1
103.6	15.8	14.5	1,3,4,& 1,3,6,& 1,3,4,6,β-D-Gal,C_1
108.1	11.7	3.7	1,3,α-L-Araf, C_1
109.4	16.9	5.5	α-L-Araf C_1→, C_1
175.1	5.1	4.0	1,(4),α-D-GlupA, C_6

4.3 Gum Arabic Fractionation Studies

4.3.1 Introduction

Although gum arabic was originally recognized as an acidic polysaccharide, it was later reported also to contain nitrogenous compounds (Anderson and Stoddart 1966) covalently linked with the polysaccharide. Gum arabic can easily be separated (by using affinity chromatography, by different molecular cut-off membranes and even by simple chemical reactions) into a number of different molecular mass fractions which also contain varying proportions of proteinaceous material. The nitrogen content can be enriched or reduced in those fractions but not completely eliminated. The amino acid composition and nitrogen content play a very important role in the emulsifying behaviour of gum arabic (Dickinson et al. 1991).

Various separation methods were applied in this section in attempts to obtain fractions showing different functional characteristics.

4.3.2 By Hydrophobic Interaction Chromatography(HIC)

4.3.2.1 Material and Experiment

HIC is an useful modern technique which separates biopolymers on the basis of their hydrophobicity.

A glass column of dimension 2.6 x 50 cm was packed with Phenyl-Sepharose CL-4B gel (Pharmacia). 35 ml of 10% gum arabic (*A. senegal*) solution in 4M NaCl was filtered through a Whatman No.1 filter paper then added to the top of the column and passed down under gravity. 4M NaCl was used as the first eluent solution. Most of the gum (ca. 85%) passed straight through the column and was collected as fraction 4M. A proportion of the gum that had been absorbed on the gel was desorbed using 3M NaCl as the second eluent solution and collected as fraction 3M. Further sequential elutions with 2M,1M, 0.1M and distilled water(0M) then gave fractions 2M, 1M, 0.1M, and 0M respectively. The collected fractions were dialysed against distilled water to eliminate chloride ions and then freeze dried. The yield of each fraction and the analytical parameters shown in Table 4.2 were determined. The total yield recovery was 95% and the nitrogen recovery was 91%.

4.3.2.2 Results and Discussion

Table 4.2 Amino acid composition (per 1000 residues) of Phenyl-Sepharose Cl-4B Gel filtration fractions for *A. senegal* (L.) Willd. gum

Amino Acids	Fractions						GA*
	4M	3M	2M	1M	0.1M	0M	
Ala	28	15	22	35	42	52	28
Arg	7	4	7	13	12	31	12
Asp	73	27	43	60	96	101	51
Cys	0	0	0	7	21	86	0
Glu	33	23	43	57	64	76	40
Gly	66	45	48	67	79	89	59
His	72	58	58	53	40	23	55
Hyp	193	395	282	138	133	33	260
Ile	7	6	9	17	22	38	12
Leu	70	71	73	79	88	85	76
Lys	31	17	36	42	38	50	30
Met	0	0	0	2	1	3	2
Phe	20	27	40	45	71	55	35
Pro	92	63	72	85	48	64	70
Ser	177	142	152	152	107	74	144
Thr	92	80	77	78	61	51	80
Tyr	7	6	7	21	10	25	14
Val	32	21	31	49	67	64	32
NCF	6.23	6.65	6.51	6.36	6.59	6.40	6.47
Yield mg	3000	180	118	17	34	130	3500
Yield%	86	5.1	3.4	0.5	1.0	3.7	n.d.
N%	0.26	1.75	2.1	2.4	3.3	1.45	0.34
Protein%	1.62	11.6	13.7	15.3	21.7	9.30	2.20
Mw x10^5	7.2-22	63	14	24	42	1.4	12
$[\alpha]_D$	-30°	-34°	-36°	-36°	-33°	-25°	-31°
[η]ml/g	14	36	20	25	34	5	16
E.A.(1%)	0.86	1.48	1.45	1.24	n.d.	0.91	1.45
ES_{30}%	16	57	70	65	n.d.	25	52

* Natural gum arabic (*Acacia senegal*) n.d.=not determined

Fraction 4M (86%) contains the major components of the natural gum arabic except for those adsorbed by the Phenyl-Sepharose gel. Its emulsifying stability was drastically decreased although the total protein content, the amino acid composition, and the specific rotation were not significantly changed. This indicates that the small proportion of the hydrophobic components adsorbed by the gel are important for the emulsification function of the gum. With the molecular mass and the intrinsic viscosities of these fractions varying, but the specific rotation values varying only slightly, it appears that the sugar linkage environments are very similar in those

fractions. On the other hand, the amino acid compositions showed marked changes from fraction 3M to 0M. The contents of Ala, Arg, Asp, Cys, Glu, Gly, Ile, Lys, Tyr, Val all increase progressively; whilst the Hyp, Ser, Thr decrease. The more hydrophobic the component, the more likely is it to be adsorbed onto this gel. Fractions 1M, 0.1M and 0M are hydrophobic and such fractions may be located at peripheral positions in the macromolecular structure since it has been shown that the enriched amino acids of the galactan core are Hyp, Thr, Ser (Anderson and McDougal 1987a). The fraction with the best emulsifying behaviour was fraction 2M whose analytical parameters and amino acid composition were very similar to whole gum arabic, the nitrogen content being the main difference between fraction 2M and GA. It has been established (Dickinson et al. 1988) that the emulsifying properties are dependent not only in the total protein content but also its distribution between low- and high- molecular weight fractions, and on the molecular accessability of the protein/peptide for the adsorption processes involved.

Previous researchers (Randall et al. 1989) concluded that gum arabic consists of a continuum of different components, viz. an arabinogalactan, arabinogalactan/complex, and a glycoprotein but no separate fractions were isolated. More fractions can however be isolated if a wider range of concentrations, or less drastically differing concentrations, of the desorbing eluent solution are employed. It therefore appears that the gum arabic macromolecules incorporate a range of hydrophobic components whose amino acid compositions differ although the polysaccharide components appear to remain fairly constant.

4.3.3 By Ultrafiltration Chromatography(UFC)

Ultrafiltration(UF) involves selective rejection of solution by convective solvent flow through an anisotropic membrane (or hollow fibres). Solutes, colloids or particles of dimensions larger than the specified membrane "cut-off" are quantitatively retained in solution, while those smaller than the pores pass with solvent through the membrane's substructure.

4.3.3.1 Material and Experiment

An ultrafiltration device fitted with a cellophane membrane (molecular cut-off 14,000 daltons) contained 400 ml of 5% gum arabic (*A. senegal*) solution; 35 lb/in^2 pressure on the top of the solution and vacuum underneath were applied with continuous stirring. The solution which passed through the membrane was collected as fraction <14 and the retained fraction was collected as fraction >14 after two days.

A Hollow Fibre Cartridge (Amicon Corporation, U.S.A.) of nominal cut-off 100,000 molecular weight was used by continuously pumping 400ml of 5% gum arabic solution into it. The material which passed through the pores of the fine cylindrical tubes was collected as fraction <100 and the residual circulating solution was collected as fraction >100 after two days. All the fractions were freeze-dried and recoveries were determined.

4.3.3.2 Results and Discussion

Table 4.3 Amino acid composition and other analytical data for *Acacia senegal* gum fractions separated by ultrafiltration

Amino Acids	Fractions				
	<14	>14	GA	>100	<100
Ala	41	27	28	26	60
Arg	12	10	12	10	16
Asp	75	49	51	44	133
Cys	22	0	0	0	15
Glu	59	38	40	38	76
Gly	75	56	59	53	76
His	39	56	55	57	15
Hyp	198	266	260	282	49
Ile	17	11	12	12	30
Leu	67	79	76	76	73
Lys	43	31	30	28	43
Met	3	2	2	1	8
Phe	47	35	35	33	65
Pro	65	69	70	69	90
Ser	111	145	144	149	85
Thr	65	81	80	81	70
Tyr	19	13	14	12	23
Val	42	32	32	29	73
NCF	6.54	6.70	6.47	6.53	6.77
Yield%	4	96	-	90	10
N%	0.69	0.33	0.34	0.35	0.45
Protein%	4.5	2.2	2.2	2.3	3.0
$[\alpha]_D$	-16°	-31°	-31°	-29°	-13°
$[\eta]$ ml/g	4.5	16.0	16.0	16.8	6.5
Sugar ratio after hydrolysis%					
Gal	20	48	46	48	39
Ara	30	38	38	38	34
Rha	50	14	16	14	27

Table 4.3 shows the amino acid composition and other characteristics of those

fractions. There are no distinct differences between fraction >14 and >100, which were the major fractions obtained, and whole gum arabic(GA) so far as both the sugar ratio and amino acid composition were concerned. But the data for the two smaller molecular mass fractions (<14 and <100) have neutral sugar ratios that are changed distinctly in comparison with that of the whole gum, the rhamnose content increasing from 16% up to 50%. In addition, the amino acid compositions show that the His, Hyp, Ser, Thr, Leu contents in fractions <14 and <100 were smaller than in the whole gum. Those amino acids have long been known to be involved in covalent linkages between the polysaccharide and protein moieties in glycoproteins and proteoglycans (Lamport 1967; Selvendran and O'Neill 1982; Fincher et al. 1983), while the other amino acid contents increased from 38% to 52% and 70% respectively in fractions <14 and <100. The nitrogen content also increased in these small molecular weight fractions. This suggests that these small amounts of low molecular weight fractions have higher rhamnose, higher nitrogen, and smaller Hyp, Ser, Thr, His contents. These fractions are suspected to play a role in the emulsifying function of gum arabic. By sequential Smith-degradations, the core structure of gum arabic was shown to consist of galactose and Hyp, Ser, Thr, Leu, Pro (Anderson and McDougal 1987a) and it has long been established from classical studies that all of the rhamnose in gum arabic is chain-terminal. It may therefore be possible that those small molecular mass fractions may represent the structural features of the peripheral regions of the structure of the gum arabic macromolecules.

4.3.4 By Chemical Separation

The purification of gum arabic by precipitation with acidic alcohol or acetone has been long known. Although the behaviour of gums with various chemical reagents was summarized (Glicksman 1970), the mechanisms involved were not fully understood. During the progress of this study, it was noticed that gum arabic solutions sometimes form a flocculent precipitate after the addition of ferric chloride solution, as is required in the official tannin-bearing test method for gum arabic (FAO 1990).

4.3.4.1 Material and Experiment

A series of four simple experiments was undertaken. (1) To 1% gum arabic solution was added 1ml 0.2M ferric chloride. A yellowish cloudy flocculent solution

formed immediately; after centrifugation, the sediment was obtained as Fraction #a. (2) A proteinaceous material extraction solvent (phenol:acetic acid:water=1:1:1) (Stanley et al. 1968) was used to dissolve gum arabic (5%) overnight; sufficient ethanol was then added to precipitate the gum. After centrifugation at 4000 rpm for 5 mins, the supernatant was freeze-dried as Fraction #b. (3) 20 grams Ethylenediamine tetracetic acid was dissolved in 600 ml 5% gum arabic solution overnight. sufficient ethanol was then added to precipitate the gum. After centrifugation, the supernatant was collected and freeze-dried as Fraction #c. (4) Fraction #d was obtained simply by setting 10% gum arabic solution aside for one month in an air-tight jar; the flocculent sediment that formed was recovered.

4.3.4.2 Results and Discussion

Table 4.4 Amino acid composition (per 1000 residues) for *A. senegal* gum fractions

Amino Acids	GA	Fractions			
		#a	#b	#c	#d
Ala	28	51	43	63	81
Arg	12	12	15	26	31
Asp	51	82	90	95	102
Cys	0	0	29	27	0
Glu	40	76	76	64	92
Gly	59	70	71	106	87
His	55	43	30	24	33
Hyp	260	169	147	119	81
Ile	12	13	23	22	36
Leu	76	71	74	56	86
Lys	30	33	44	37	47
Met	2	0	3	8	11
Phe	35	44	57	29	38
Pro	70	45	81	53	57
Ser	144	120	90	130	77
Thr	80	65	48	64	53
Tyr	14	50	20	29	24
Val	32	56	57	48	64
NCF	6.47	6.68	6.67	6.32	6.30
N%	0.34	0.37	1.5	0.39	1.1
Yield%	-	<5	<2	<2	<3

Table 4.4 shows the amino acid composition of those four fractions obtained from whole gum arabic. It was found that all the fractions were protein-enriched. (1) The sugar composition of fraction #a was Gal:Ara:Rha:= 37:34:29, while the sugar ratio

for the supernatant was 48:36:16 i.e. nearly the same as that for whole GA. (2) Phenol has long been known to be a good and rather selective solvent for extracting polypeptide material from biological sources (Pusztai 1966). The phenol-acidic acid-water solvent has been shown to be very effective for dissociating proteins, but does not cleave peptide bonds (Van Sumere et al. 1975). The sugar composition of Fraction #b consisted of Gal:Ara:Rha=24:42:34, therefore again showing a higher rhamnose content and with lower Hyp, Ser, Thr, Leu, His contents in the amino acid composition. (3) and (4) Similar properties are shown by Fractions #c and #d. Although different chemical methods were applied to obtain those four fractions, they all contain higher rhamnose, and enriched protein of altered composition. These fractions are possibly loosely located at the peripheral positions of the gum structure because of the mild chemical treatments leading to their release.

4.3.5 By Enzymatic degradation

Enzymes have been used to degrade polysaccharides to obtain structural information. They have been applied in many fields, such as for determining the degree of polymerization of glucans and xylans (Sturgeon 1980), for analysing acidic glycosaminoglycans (Murata 1980) and for modifying natural seed gums (Reid et al. 1987). Enzymatic hydrolysis of peach gum (*Prunus persica*) yielded a number of oligosaccharides that demonstrated the presence of (1→3) and (1→6) linkages within chains of β-D-Gal units, and a (1→3) linkage between α-D-Galp (Kardosova et al. 1979). Gum arabic solution (considered as arabinogalactan-protein complexes) treated with protease type E (Sigma Chemical Ltd) showed modified GPC profiles indicating that the molecular mass changed from 4.83×10^5 to 3.92×10^5 after 72 hours enzymatic degradation (Randall et al. 1988). Similar work led the workers to propose a structure wherein varying numbers of polysaccharide units of Mw ca. 2×10^5 are linked to the protein core of a gum arabic structure (Connolly et al. 1988). But the nature of the proteins located on the periphery, which may play an important role in the emulsification functionality of gum arabic, have not been reported.

4.3.5.1 Material and Experimental Method

1 ml Viscozyme 120L(NOVO, Denmark), a carbohydrase complex, was added to 400 ml of 10% gum arabic (*A. senegal*) solution (natural pH); the solution was allowed to stand at 40°C for 96 hours, a 20 ml aliquot being abstracted after each of 1, 3, 18 and 48 hours. The 20 ml aliquot was heated at 75°C for 5 mins in order to inactivate the enzyme each time and was dialysed against distilled water (regular

changes) for 5 hours, using a 12,000 -14,000 molecular cut-off membrane. The dialysates were collected and freeze-dried to give D_1, D_3, D_{18} and D_{48}. The remaining solutions were continuously dialysed against running water for a further two days and collected as before,giving S_1, S_3, S_{18} and S_{48}. A_{free} is the material remaining in the incubation gum solution after 96 hours (**without 6 N HCl hydrolysis**). En is the composition of Viscozyme 120L.

4.3.5.2 Results and Discussion

Table 4.5 Amino acid composition (per 1000 residues) for enzyme-degraded fractions

Amino	Fractions										
Acids	S_1	S_3	S_{18}	S_{48}	D_1	D_3	D_{18}	D_{48}	A_{free}	GA	En
Ala	28	29	26	28	92	65	82	60	238	28	76
Arg	12	9	10	9	0	4	2	5	0	12	20
Asp	51	51	52	51	102	144	137	113	14	51	134
Cys	0	0	0	0	44	12	0	0	33	0	70
Glu	40	40	38	38	136	137	157	128	31	40	82
Gly	59	59	59	61	116	100	108	89	98	59	101
His	55	52	59	56	12	11	11	11	70	55	14
Hyp	260	275	289	296	28	44	76	116	15	260	0
Ile	12	12	10	12	35	35	32	27	17	12	43
Leu	76	77	74	70	44	58	55	43	61	76	54
Lys	30	24	29	21	43	22	22	44	32	30	37
Met	2	2	1	1	6	8	10	5	3	2	7
Phe	35	31	33	31	25	40	40	32	59	35	34
Pro	70	70	75	76	81	61	68	84	156	70	34
Ser	144	137	153	116	82	86	73	80	66	144	99
Thr	80	84	45	84	53	78	57	64	2	80	95
Tyr	14	15	15	16	31	33	12	39	20	14	41
Val	32	33	32	34	52	62	58	60	85	32	59
NCF	6.47	6.56	6.47	6.56	6.81	7.06	7.02	6.99	5.80	6.47	6.58
N%	0.37	0.37	0.37	0.37	0.60	0.64	0.70	0.62	n.d.	0.34	1.37
[η]	17	15	13.5	12	n.d.	n.d.	n.d.	n.d.	n.d.	16	18
Sugar composition after hydrolysis%											
Gal	n.d.	n.d.	n.d.	n.d.	tr	10	20	28	25	46	tr
Ara	n.d.	n.d.	n.d.	n.d.	20	18	30	35	35	38	0
Rha	n.d.	n.d.	n.d.	n.d.	80	72	50	37	40	16	0
Mw x 10^4	59	47	38	31	n.d.	n.d.	n.d.	n.d.	n.d.	120	n.d.

n.d.= Not determined because of limited amounts of material.

Table 4.5 shows the amino acid compositions and some characters of those enzyme-degraded fractions. S_1, S_3, S_{18} and S_{48} showed no distinct differences in

amino acids composition from GA, although the intrinsic viscosity values decreased progressively. The estimated average molecular mass (Mw) was calculated from the Mark-Houwink equation $[\eta]=K'Mw^a$ assuming K'=0.013 and a=0.54 (Anderson and Rahman 1967). After 48 hours enzymatic degradation, the molecular mass of S fractions fell from original $5.9x10^5$ to $3.1x10^5$ which was in reasonable agreement with the results obtained for pronase-treated gum arabic (Connolly et al. 1988). The proportional ratios between the S and D fractions after 1, 3, 18, and 48 hours were 99.5:0.5, 94:6, 87:13 and 80:20. In contrast, the D fractions showed large differences in amino acid and sugar compositions; Ala, Asp, Cys, Glu, Gly, Ile, Tyr and Val were enriched while Arg, His, Hyp, Leu, Ser and Thr were reduced. In terms of the sugar composition, the rhamnose content was reduced in the D fractions as the degradation progressed but all fractions contain much more rhamnose than the original gum arabic. By carefully examining and comparing the data for A_{free} with that for GA, A_{free} shows significant differences and its amino acid data represent the free amino acid composition (without 6N HCl hydrolysis) after 96 hours of enzyme treatment. Generally, terminal amino acids are more easily released from peptide chains and Ala clearly dominates the composition (24%, in A_{free} i.e. 8 times more than in GA) with Pro, Val and Cys also suggested as mainly terminal amino acids. On the other hand, Hyp and Ser contents in the D fractions are much smaller than in the whole gum. Hyp and Thr are very low in A_{free}, decreasing from 34% in GA to only 1.7% in A_{free}. This indicates that these two amino acids are in peripheral positions; Hyp and Ser have already been shown to be mainly associated with the core galactan (Anderson and McDougal 1987a). Free sugars were also released by the enzyme, and again rhamnose is involved to the greatest extent.

4.4 Studies of Gum Arabic from Uganda

4.4.1 Introduction

A good *Acacia senegal* tree yields only about 1 kg of gum per season and hence each tonne of gum must be a mixture of the gum from at least 1000 trees. Large commercial consignments of gum arabic therefore contain many individual collections, possibly from a range of botanical provenances, with these collections being mainly from Sudan (85%), but also possibly from Nigeria, Kenya, Uganda and other Sahelian countries. This mode of collection of gum arabic therefore produces a trading supply of gum which is liable to vary in purity and identity. For such a complex natural product, it is not surprising that seasonal and geographical variations

in composition have long been established (Anderson et al. 1968a). All that can be done to satisfy modern regulatory demands for specifications of identity and purity is to try to establish the range and average values for each analytical parameter from data for an adequate number of representative samples. This has been done for the polysaccharide parameters (Anderson et al. 1983a) and amino acid composition of gum arabic (Anderson et al. 1985a) for an adequate number of Sudanese and Nigerian samples, but not yet for other, minor, producing countries.

In this study, gum arabic samples from Uganda have been characterized. It is important to have the analytical parameters for gum arabic from different regions to check whether they lie outside of the range of variation established for Sudanese and Nigerian samples, which together account for over 90% of world production.

4.4.2 Origin of Gum Samples

Seven natural exudate gum arabic samples from the Steppes and *Acacia* woodlands of North-east Uganda bordering southern Sudan, which represents Uganda's driest regions, were collected by the District Forest Officer of Karamoja Region in collaboration with Uganda's Ministry of Environmental Protection. Each sample comprised clean, pale-coloured gum of excellent solubility. The analytical data obtained are shown in Table 4.6(a) and 4.6(b), which also includes the mean data established for large number of Sudanese samples (GA_{su}) (Anderson et al.1990).

In addition, a further five *A. senegal* and two *A. seyal* gum samples were subsequently obtained from Uganda, along with the soil upon which those trees were growing, to facilitate the the analysis of their cationic compositions by X-ray fluorescence methods. *A. senegal* samples 1N, 2N and 10N were collected from trees at Nakicumet from soil of a dark-brown colour; *A. senegal* sample 11K was collected at Kokers (red-brown soil); *A. senegal* L3 and *A. seyal* L5 and L6 were collected at Longiro, where the soil is black.

4.4.3 Results and Discussion

Table 4.6(a) contains the analytical data, including emulsification functionality data, (EA & ES), obtained for 7 *A. senegal* gum samples from Uganda. There are only small analytical differences between the samples. The nitrogen content (0.27%) of the samples is lower than the average for Sudanese samples (0.34%), and lies at the bottom of the range (0.27 - 0.39%) quoted (FAO 1990) in the revised specification. The methoxyl content of the Ugandan samples is also lower than average but the

specific rotations fall within the limits of -26° to -34° (FAO 1990). One Ugandan sample is slightly more viscous (19ml/g) and one is considerably less viscous (12ml/g) than found on average (17ml/g) for Sudanese samples. The Ugandan samples have similar uronic acid contents and sugar composition to Sudanese samples, except for sample 7 which is slightly more acidic and sample 4 which tends to have a slightly low rhamnose content (9%).

Table 4.6(a) Analytical data for *A. senegal* gum samples from Uganda

	Uganda gum samples							
	1	2	3	4	5	6	7	GA_{su}
H_2O%	14.2	14.8	13.9	13.2	15.2	14.7	14.2	13
Ash%	3.2	3.5	3.9	4.5	3.9	3.9	4.5	2.6
N%	0.27	0.27	0.28	0.28	0.27	0.27	0.28	0.34
NCF	6.50	6.50	6.68	6.60	6.62	6.77	6.62	6.62
Protein%	1.8	1.8	1.9	1.8	1.8	1.8	1.9	2.3
Methoxyl%	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.25
$[\alpha]_D$	-32°	-30°	-32°	-26°	-34°	-34°	-32°	-30°
[η]ml/g	15.0	14.5	12.0	13.7	16.5	15.0	19.0	16.0
E.Wt[a]	1030	1060	1030	1200	1070	1180	930	1050
UAA	17	17	17	15	16	15	19	17
EA	1.65	1.66	1.38	1.54	1.55	1.43	1.51	1.60
ES_{30}%	74	80	40	72	66	29	56	95
Sugar composition after hydrolysis%								
MGUA[b]	1	1	1	1	1	1	1	1.5
GUA	16	16	16	14	15	14	18	16
Gal	48	48	44	52	46	44	45	44
Ara	22	24	28	24	24	27	24	25
Rha	13	11	11	9	14	14	12	14

a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).

The emulsification functionality of those samples, estimated as Emulsification Activity(EA) and Emulsification Stability(ES) according to the method devised by James and Patel (James and Patel 1988), varies widely to an extent similar to that observed for Kenyan samples (Anderson and Wang 1990). This rapid mixing method of assessing emulsion functionality at single wavelength has limitations (Dickinson and Stainsby 1988), but such a method has been used meaningfully (Randall et al. 1988) to assess relative emulsion stability, and gave comparable results for the emulsification efficiencies found in a detailed, fundamental study of the gums from different *Acacia* species (Dickinson et al. 1988). It has been concluded that the nature and distribution of the proteinaceous components of gum arabic are important

rather than their overall amount (Dickinson et. al. 1991), but the galactose/arabinose ratios, rhamnose and nitrogen contents, and viscosity also appear to be involved in emulsification functionality.

Table 4.6(b) Analytical data for *A. senegal* gum samples from Uganda

	Uganda gum samples							
	1	2	3	4	5	6	7	GA_{su}
Amino acid composition (per 1000 residues)								
Ala	37	28	45	31	39	46	41	27
Arg	19	10	14	11	15	13	13	13
Asp	67	49	76	56	70	72	63	68
Cys	51	0	0	0	12	13	13	2
Glu	62	41	61	46	58	55	53	42
Gly	54	40	57	47	57	58	51	50
His	41	51	38	51	39	34	40	44
Hyp	179	266	216	257	232	253	240	304
Ile	18	10	16	12	17	17	15	12
Leu	72	65	67	70	69	63	66	66
Lys	42	56	38	30	44	42	54	25
Met	2	0	2	2	1	2	1	2
Phe	44	40	53	45	48	54	49	33
Pro	64	70	65	82	65	49	64	63
Ser	113	144	109	135	105	99	110	129
Thr	71	80	65	74	62	55	64	68
Tyr	16	17	27	15	20	23	25	14
Val	48	33	49	36	48	49	41	35
NCF	6.50	6.50	6.68	6.60	6.62	6.72	6.62	6.62
The cationic composition of the ash(ppm)								
Al	1944	2214	1269	3386	1468	1003	949	190
Ca	107490	186206	140500	144799	225723	138629	112605	256000
Cd	0	0	0	0	0	0	0	0
Co	12	7	0	10	3	19	0	<1
Cr	661	1357	706	1562	1174	640	868	47
Cu	1021	1739	1269	2828	546	455	868	52
Fe	1508	1866	830	2491	911	734	982	128
Mg	34047	35205	35352	40527	52510	38162	23493	38000
Mn	68	77	46	117	83	69	58	100
Na	8755	1400	4759	9287	10229	12072	1458	9400
Ni	18	24	13	30	12	12	6	10
Pb	23	46	35	67	26	20	18	6
K	249027	196810	313633	261736	253682	352025	312703	237000
Zn	57	85	66	111	68	45	68	24

Table 4.6(b) shows that there are variations in the amino acid compositions of the Ugandan samples, but these are similar in extent to those recorded (Anderson et al.

1990) for Sudanese and Nigerian samples. Nevertheless, the Ugandan samples have lower Hyp content and tend to have higher proportions of Ala, Lys, Phe, Tyr and Val, i.e. of some of the amino acids with lipophilic rather than hydrophilic properties.

Table 4.7 The cationic composition(ppm) from *Acacia* gums and associated soil from Uganda by X-ray fluorescence spectrometry

Cations		*A. senegal*					*A. seyal*	
		1N	5N	10N	11K	L3	L5	L6
Al	in gum	217	260	233	405	324	111	578
	in soil	79080	101230	103860	91750	102860	107165	107300
Ba	in gum	59	33	54	17	16	116	67
	in soil	600	690	697	947	1001	947	868
Ca	in gum	9882	8075	6752	4695	4100	12089	8842
	in soil	11940	11680	11320	12520	15020	10666	10441
Cr	in gum	0.2	0.4	0.6	0	0.5	1.0	1.9
	in soil	58	66	60	50	101	139	130
Cu	in gum	1.6	1.9	2.5	2.5	0.6	2.2	1.8
	in soil	11	12	14	12	13	34	34
Fe	in gum	22	23	42	46	142	28	451
	in soil	23290	32800	34160	31330	34100	62400	61764
K	in gum	8952	12736	16119	17855	15694	2233	3668
	in soil	12960	17350	14980	26240	14016	13766	14210
Mg	in gum	1378	1140	716	948	1973	1052	557
	in soil	7290	7260	5136	6440	12700	12642	12613
Mn	in gum	3.7	4.4	2.9	2.9	4.8	2.5	6.5
	in soil	234	333	332	406	866	1435	1107
Na	in gum	34	40	26	57	56	27	118
	in soil	12230	8729	8050	10310	8990	2515	2291
Ni	in gum	2.7	0.5	0	0.4	0	0.7	0.8
	in soil	23	29	27	24	38	65	58
Pb	in gum	0.4	0	0.1	0.5	1.1	0.1	0.6
	in soil	13	14	15	15	25	22	22
Sr	in gum	115	87	72	62	42	138	132
	in soil	252	217	222	438	434	216	229
Ti	in gum	3.6	3.8	7.2	8	19	4	48
	in soil	4140	3870	4980	6400	5467	8355	7995
Zn	in gum	0.3	2.2	2.1	1.0	2.3	1.1	1.3
	in soil	26	39	38	37	50	80	79
P	in gum	11	8	9	8	6	6	37
Si	in gum	531	619	615	1084	1064	264	1402

The Ugandan samples also have a considerably lower calcium content than that reported (Anderson et al. 1990) for Sudanese samples. In addition, the Ugandan samples have much higher levels of aluminium, chromium, copper, and iron than have been found in East African (Anderson and Morrison 1989), Kenyan (Anderson

and Wang 1990) and Sudanese/Nigerian (Anderson et al. 1990) samples. Such variations in cationic composition, particularly for heavy metals for which upper limits are specified (FAO 1990), presumably reflect the abundance of particular elements in soils at different locations.

Ugandan soils are reported to be predominantly ferralites (high in iron and aluminium). Table 4.7 shows the cationic compositions of the gum samples in comparison with that of the soil upon which the gum-producing tree grew. Although there are variations in those samples, calcium and potassium are the major cations in the gums, as is cusstomary for gum arabic from other regions, followed by aluminium, silicon, barium, iron, sodium and strontium (contents between tens and hundreds ppm). In comparison with the soil cationic composition, it has been found that the gum trees actively select the amount of the absorbed cations. There are not large differences in the calcium and sodium contents of the soils, but the calcium content in the gums is at least 10 times more than the sodium content. It seems that gum trees can concentrate certain types of cations, such as calcium and potassium into the gum. This may indicate that gum trees actively take up more of those cations which are essential for some metabolic purposes of the tree and limit others which may not be essential. The cationic content of gum exudates may not be as dependent on the minerals available in soil as was once believed.

4.5 Studies of Gum Arabic from Different Regions

4.5.1 Introduction

Gum arabic is defined by the joint FAO/WHO Expert Committee as "the dried, gummy exudate from tropical and subtropical *Acacia senegal* trees" (WHO 1990). As such, it is commercially the most important of the natural exudate gums permitted as food additives. The toxicological evidence for the food safety of gum arabic derived from *Acacia senegal*(L.) Willd., as designated E414 within the EC, has been reviewed (Anderson 1986). No toxicological evidence of safety of any kind exists for gums from any other *Acacia* species, of which over 900 were recognized in 1978 (Anderson 1978) with that total now believed to be in excess of 1,100.

Since gum arabic exports provide a very important source of hard currency earnings for the Sudan and other Sahelian countries, it is therefore desirable to study "gum arabic" specimens collected in such countries in order to observe any differences in the gum quality and composition from different regions. Analytical

data for gum exudates can provide a sensitive way of contributing chemotaxonomic evidence as to botanical diversity (Anderson and Brenan 1975). Because of the geographical and climatic differences, Brenan's authoritative review (Brenan 1983) of the taxonomy of *A. senegal* identified that several *Acacia* species are closely related to, and may even be confused with *A. senegal*, which belongs to a sizeable and complex group of spicate-flowered acacias. Although even the actual number of species and/or varieties within the complex is not certain, there is no doubt (Ross 1979) that much of the variation recognizable in the field in not only taxonomically significant, but also very important and meaningful from commercial points of view.

4.5.2 Origin of Gum Samples

Two gum samples, one described as "Tanzanian gum arabic" of unspecified botanical origin and another "Kenyan gum arabic" from Marsabit National Park, Northern Kenya, were submitted for study by the Overseas Development Natural Resources Institute, UK. Gum from a single "*A. senegal*" tree was collected in December 1990 at Sadore, Niger, and a gum sample was collected by Mr. N.V.Clarke from a single "*A. senegal*" tree at Anya Hamran, 17°06'N, 54°17'E, Alt. 100m, Southern Region, Oman, in May 1991. A Sudanese commercial gum arabic sample from Kordofan, Sudan, and one of the Ugandan gum arabic samples discussed in the previous section are included for comparative purposes in Table 4.8.

4.5.3 Results and Discussion

The analytical parameters in Table 4.8 reveal distinct differences between these gum samples. Only four of the six samples (those from Kenya, Niger, Uganda and Sudan) have specific rotations which fall within the specified limits of -26° to -34°. And of these four samples, the nitrogen content of the Kenyan sample (0.70%) is well outside the range (0.27-0.39%) quoted in the revised specification (FAO 1990). *A. senegal* gum samples from Kenya always tend to have higher nitrogen contents (0.40 -0.80% dry weight basis), higher intrinsic viscosity (23 -40 ml/g), and higher amounts of acidic sugars in comparison with the values established for Sudanese *A. senegal* samples (Wang 1992). This indicated that there are different varieties of *A. senegal* tree growing in Kenya than in Sudan and this has recently been confirmed by Mr. B. Chickamai, Field Officer of the Kenya Forestry Research Institute, Nairobi, who has identified the trees in the Marsabit Area as being *A. senegal var. kerensis*, the Sudanese trees being *A. senegal var. senegal*. The slight structural differences can indeed be seen in the ^{13}C NMR spectrum of the Kenyan sample (Fig. 4.2); in

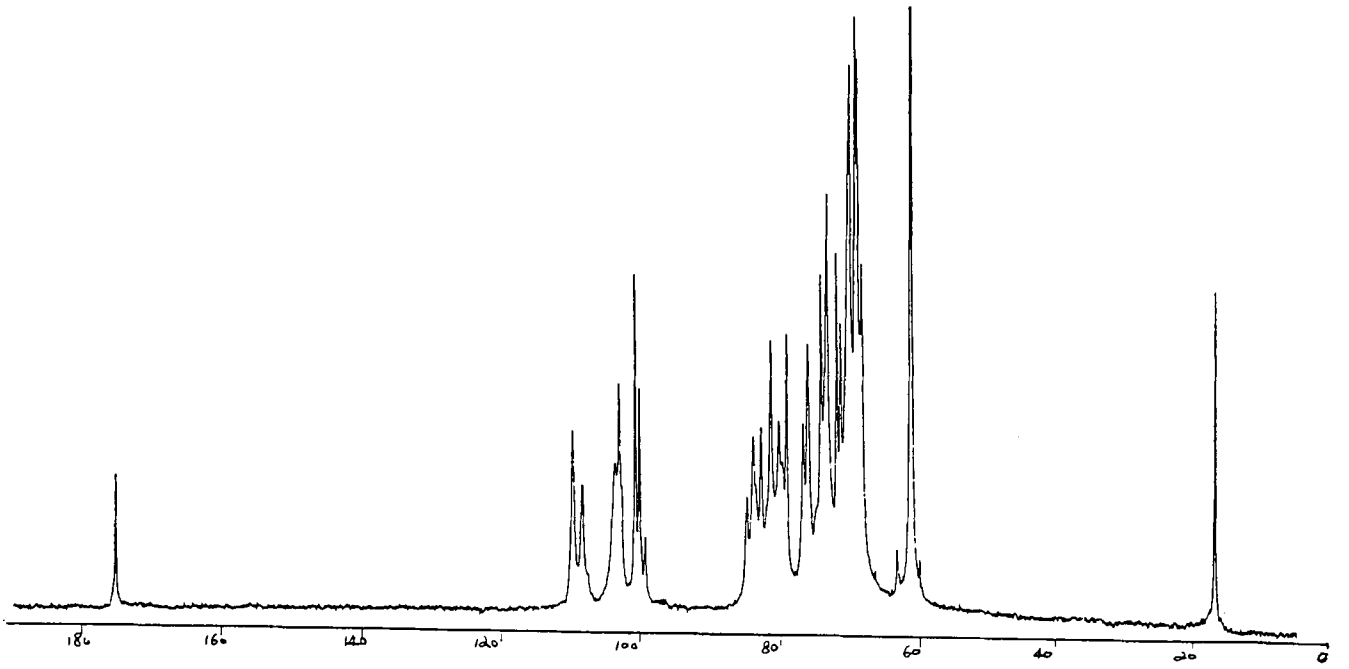

Fig. 4.1 ^{13}C NMR spectrum for *A. senegal*(L.) Willd. gum from Sudan δ(ppm)

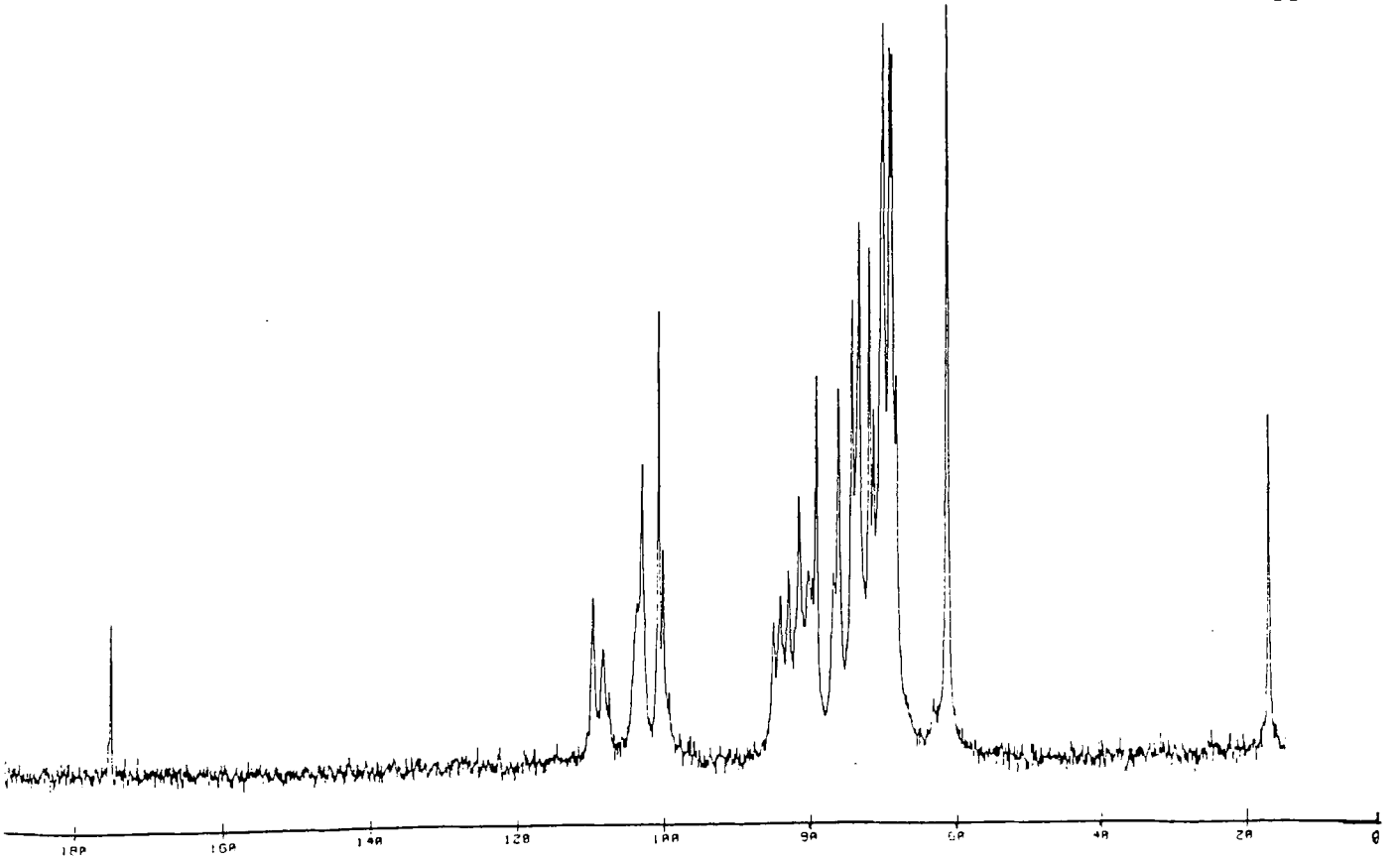

Fig. 4.2 ^{13}C NMR spectrum for "gum arabic" from Marsabit, Kenya (showing minor differences from Fig. 4.1 above) δ(ppm)

comparison with that of the Sudanese sample (Fig. 4.1) there are obviously more β Gal C_6← (or α Araf C_5←) linkages in the Kenyan sample than in the Sudanese sample, because of the reduced intensity of the 61.2 ppm peak.

Table 4.8 Analytical data for "gum arabic" samples from different locations

	Gum sample from					
	Tanzania	Kenya	Niger	Oman	Uganda	Sudan
H_2O%	12.8	13.4	12.4	17.0	15.2	13.6
Ash%	2.8	4.6	2.6	4.2	3.9	4.1
N%	0.47	0.70	0.27	0.13	0.27	0.33
NCF	6.86	6.72	6.56	6.84	6.62	6.60
Protein%	3.2	4.7	2.0	0.9	1.8	2.2
Methoxyl%	0.1	0.1	0.2	0.1	0.1	0.26
$[\alpha]_D$	-14°	-32°	-34°	-44°	-34°	-30°
[η] ml/g	10	26	16	13	17	17
E.Wt[a]	850	800	1110	990	1070	1040
U.A.A.	21	22	16	18	15	17
Sugar composition after hydrolysis%						
4-O-MGUA[b]	0.5	0.5	1	0.5	1	1.5
GUA	20.5	21.5	15	17	15	15.5
Gal	56	44	45	40	46	46
Ara	16	22	25	27	24	24
Rha	7	12	14	16	14	13
Amino acid composition (per 1000 residues)						
Ala	46	25	36	33	39	28
Arg	0	0	13	12	15	5
Asp	60	39	51	49	70	50
Cys	0	0	1	0	12	0
Glu	32	22	36	31	58	29
Gly	42	38	56	27	57	41
His	41	37	47	33	39	44
Hyp	324	456	293	341	232	328
Ile	15	0	10	11	17	12
Leu	60	47	69	48	69	67
Lys	21	0	28	33	44	23
Met	0	0	2	1	1	1
Phe	21	11	31	18	48	22
Pro	68	138	67	78	65	88
Ser	121	100	136	144	105	136
Thr	61	65	74	61	62	76
Tyr	16	2	21	40	20	10
Val	54	20	35	40	48	36
NCF	6.86	7.01	6.56	6.84	6.62	6.60

a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).

The data in Table 4.8 show that the gum samples from Niger and Uganda comply with the requirements of the revised FAO specification. Additionally, ^{13}C NMR spectra (Figs. 4.3 and 4.4) show that those samples are similar structurally to Sudanese gum arabic although fine-structure differences do exist. However, their nitrogen contents just fall within the lower limit of the specification (0.27 -0.39%).

In contrast, the specific rotations of the samples from Tanzania and Oman fall well outside the quoted specification (FAO 1990) for food grade gum arabic, and their intrinsic viscosities are lower than those for Sudanese samples. Additionally, the Tanzanian sample has a higher acidic content (21%), but a much lower rhamnose content (7%). The Oman sample contained slightly more rhamnose than Sudanese samples and was higher in Tyr and lower in Gly in its amino acid composition.

Table 4.9 The chemical shift and intensity differences shown by ^{13}C NMR spectra for "gum arabic" samples from different locations

	Gum sample from						
δppm	Tanzania	Kenya	Niger	Oman	Uganda	Sudan	C_1
99.3	5	2.5	2.3	2.6	2.4	2.1	β-Arap
100.0	2.5	7	5.9	0	7.0	6.9	α-GlupA
100.6	5.9	12	10.1	17.6	12.7	10.7	α-Rha
101.5	5.6	0	0	8.7	0	0	β-Araf
102.5	5.3	5.2	4.4	7.5	8.8	3.7	β-GlupA
102.9	-	10	7.1	8.1	6.8	7.0	β-Gal
103.6	6.0	5.3	4.4	7.0	6.5	4.5	β-Gal
108.1	1.6	3.9	3.7	5.1	5.6	3.7	α-Araf
109.4	7.8	5.5	6.1	4.6	7.5	5.5	α-Araf
109.9	0	0	0	4.7	0	0	α-Araf
61.2	22	25*	25*	14	25*	25*	β-GpC_6 α-AfC_5
62.9	5.6	0	0	10.9	0	0	β-AfC_5
63.2	-	2.0	1.7	0	2.5	1.7	β-ApC_5

* The main peak
- Peak overlapped

The ^{13}C NMR spectra for these samples (from Figs. 4.1 to 4.6) reveal the distinct structural differences in the Tanzanian (Fig. 4.6) and Oman samples (Fig. 4.5). The selected ^{13}C NMR data (Table 4.9) reveal that the samples from Kenya, Niger, Uganda and Sudan show remarkable constancy in the locations (ppm) of their major C_5 or C_6 and C_1 resonances, the origins of which are established in Chapter 3. There are, nevertheless, small spectral variations which reveal that fine structural

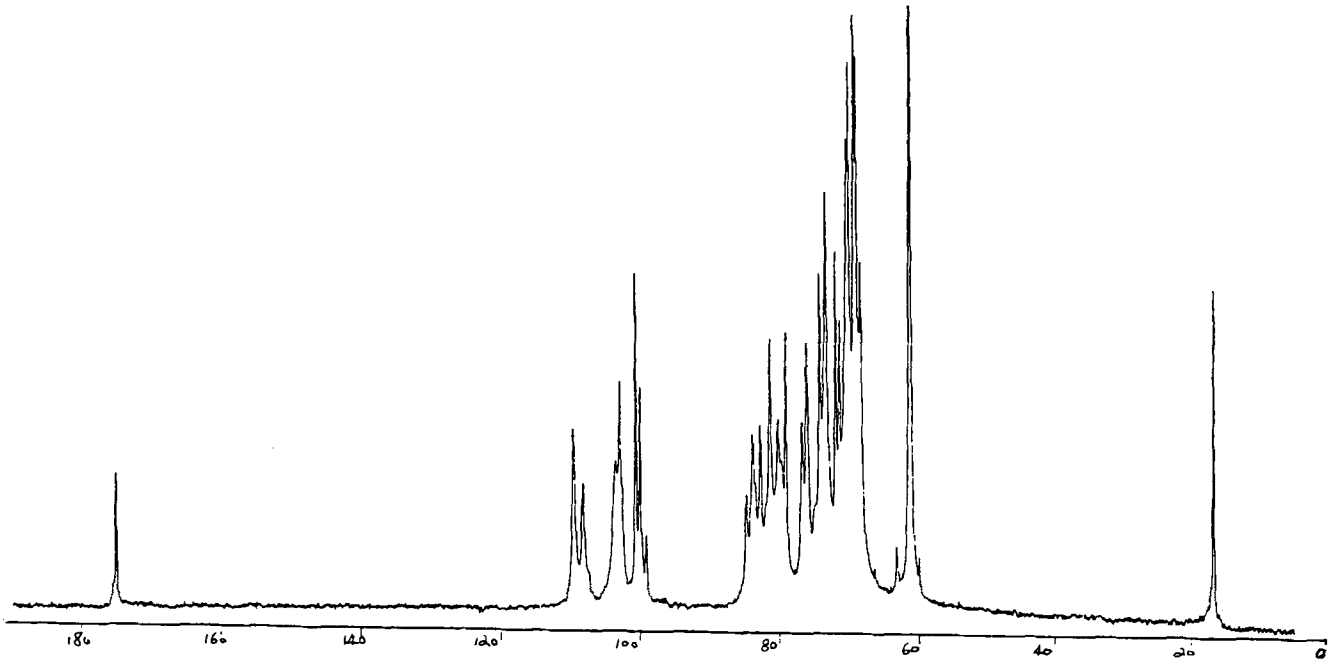

Fig. 4.1 ^{13}C NMR spectrum for *A. senegal*(L.) Willd. gum from Sudan δ(ppm)

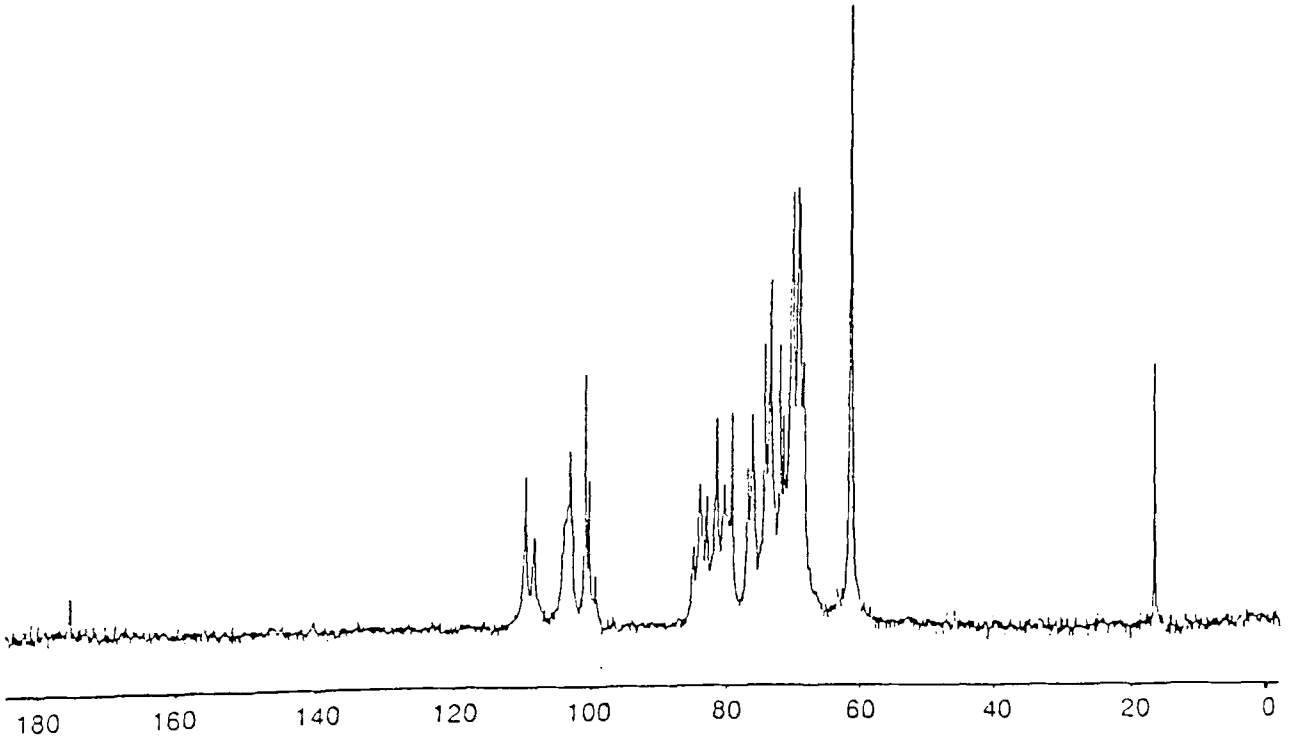

Fig. 4.3 ^{13}C NMR spectrum for *A. senegal* gum from Sadore, Niger (showing close agreement with Fig. 4.1 above) δ(ppm)

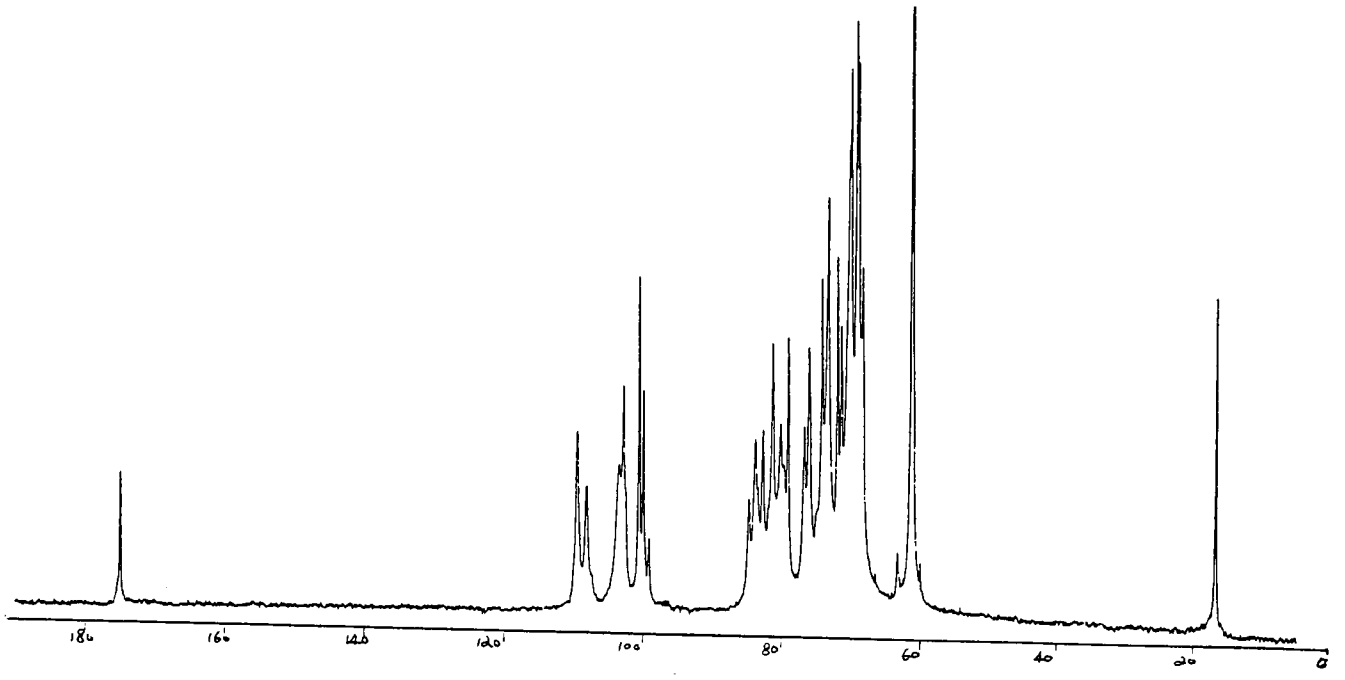

Fig. 4.1 ^{13}C NMR spectrum for *A. senegal*(L.) Willd. gum from Sudan δ(ppm)

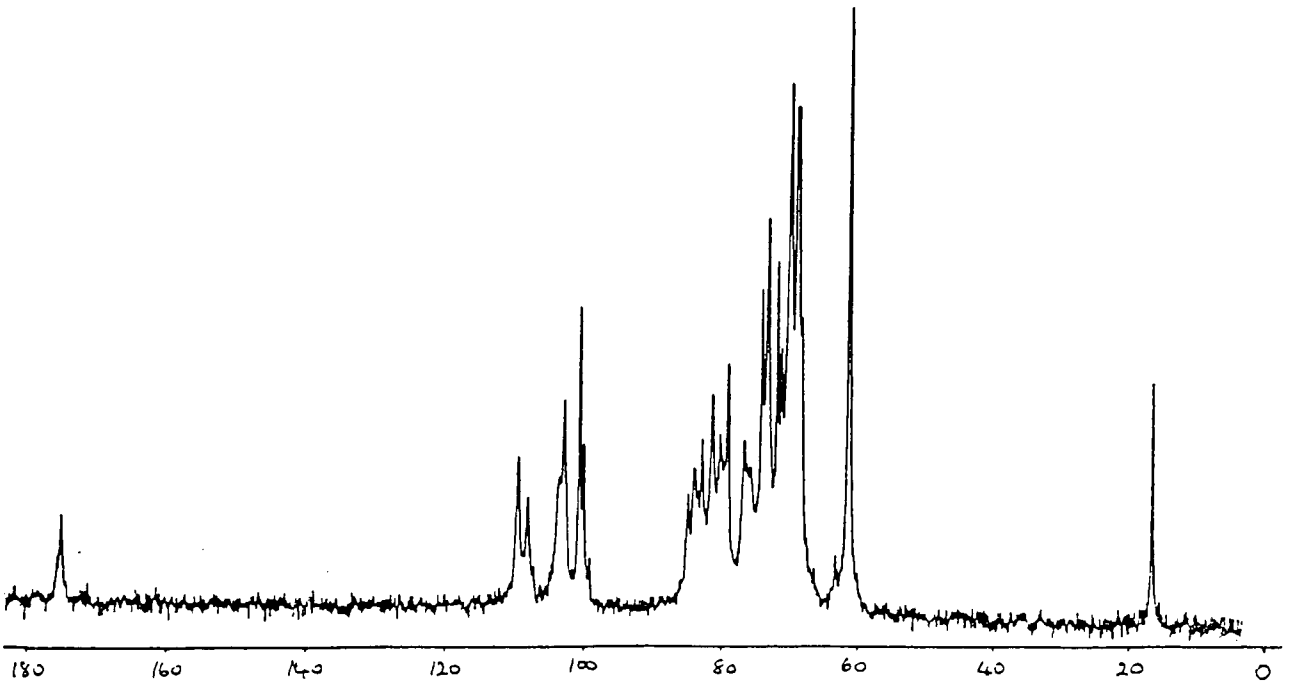

Fig. 4.4 ^{13}C NMR spectrum for *A. senegal* gum from Karamoja, Uganda (showing minor differences from Fig. 4.1 above) δ(ppm)

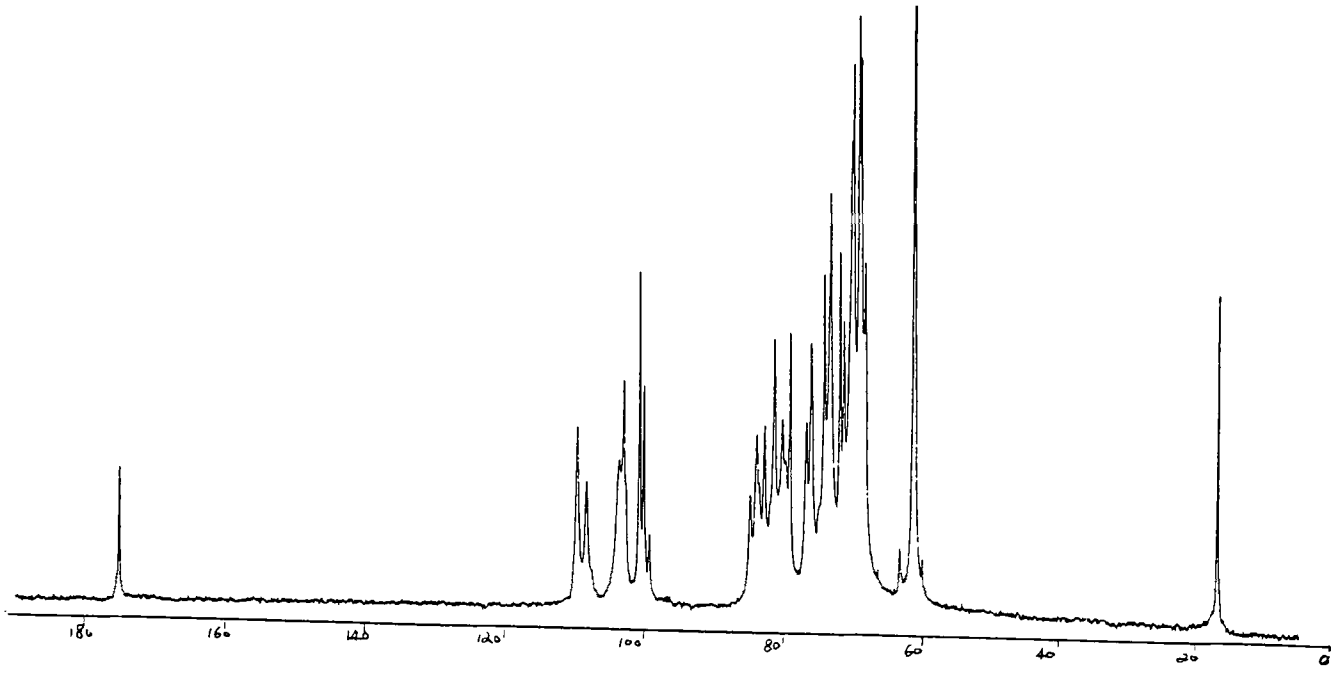

Fig. 4.1 ^{13}C NMR spectrum for *A. senegal*(L.) Willd. gum from Sudan δ(ppm)

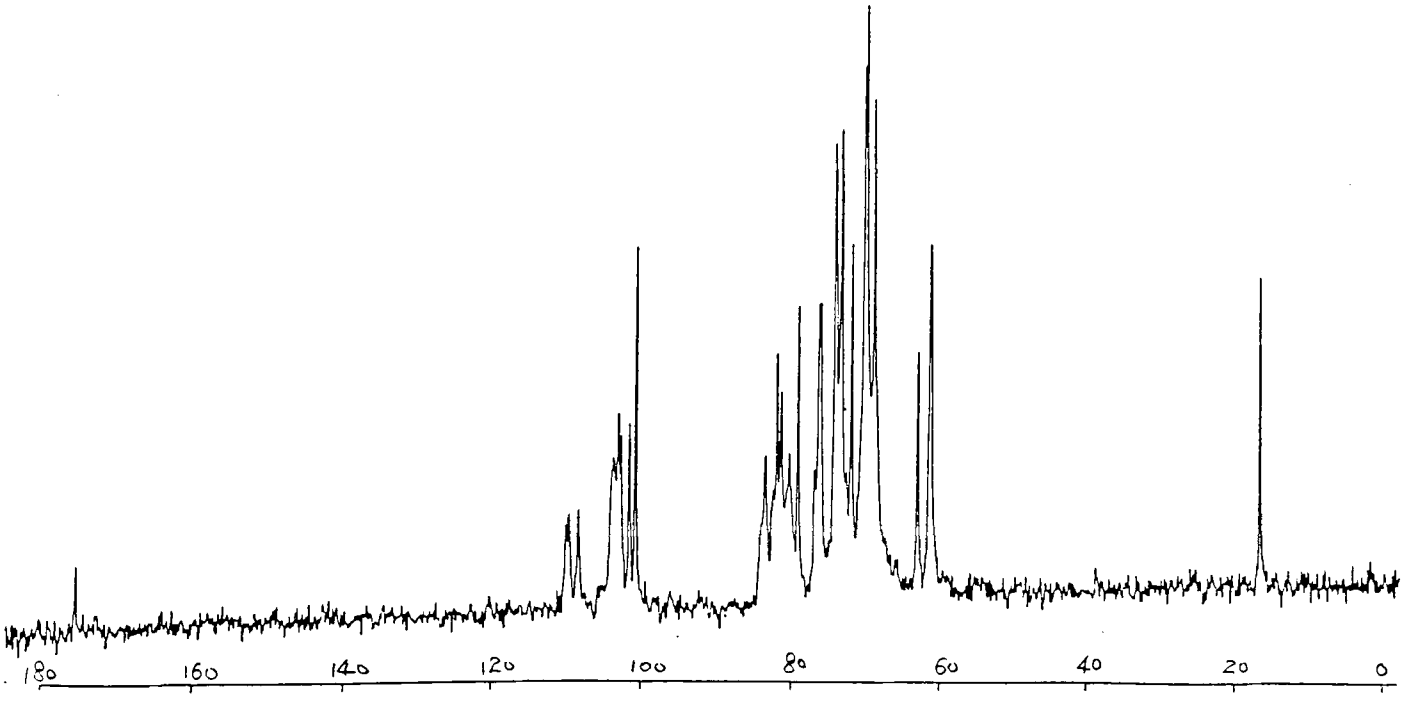

Fig. 4.5 ^{13}C NMR spectrum for "*A. senegal*" gum from Oman (showing major differences from Fig. 4.1 above) δ(ppm)

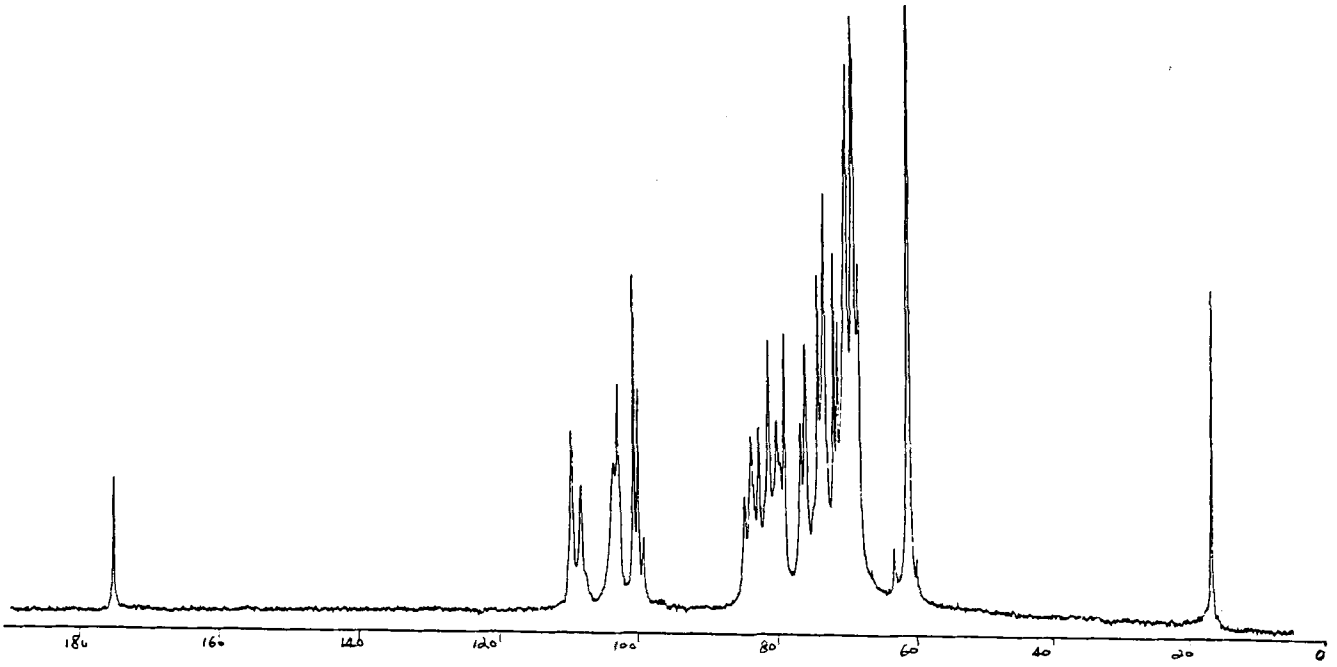

Fig. 4.1 ^{13}C NMR spectrum for *A. senegal*(L.) Willd. gum from Sudan δ(ppm)

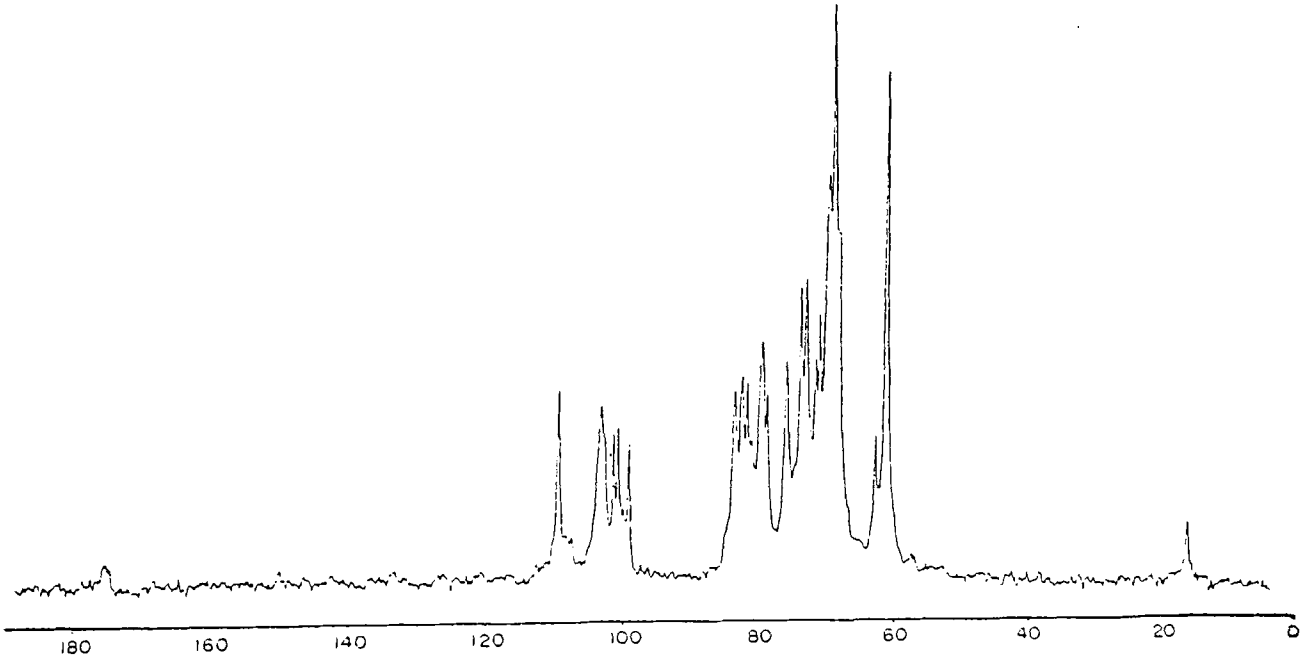

Fig. 4.6 ^{13}C NMR spectrum for "gum arabic" from Tanzania (showing major differences from Fig. 4.1 above) δ(ppm)

differences exist between those samples as must be expected from the seasonal, geographic, and taxonomic factors involved (Brenan 1983; Ross 1979). In contrast, β-Araf has never been found in those Sudanese and other gum arabic samples whose analytical data meet the specifications required, whereas it occurs in the samples from Tanzania and Oman (β-Araf C_1 at 101.5 ppm and C_5 at 62.9 ppm, Figs. 4.6 and 4.5). In addition, the widely differing intensities of other resonances reflect extensive fine structural differences.

Consideration of the comparative data in Tables 4.8 and 4.9 leads to the conclusion that the structural differences between the gums from Tanzania and Oman and those from the Sudan are sufficiently extensive to indicate that they would differ in terms of their functional performance. This would be of importance in assessing the suitability of these gums for technological applications should they ever become available in commercial quantities from agroforestry developments which are under consideration in these countries. It has been suggested to international botanical authorities that the chemical evidence suggests that the trees in Oman and Tanzania may have been incorrectly identified as "*Acacia senegal*" as has indeed been suspected on other grounds (atypical morphological features of the trees etc.).

Chapter 5
Studies of Acacia Gum Species

5.1 Introduction

The gums exuded from species of the genus *Acacia* have been important commercial materials since ancient time. Some of their industrial uses and applications were summarised (Glicksman 1970; Whistler 1973; Davidson 1980; Sandford and Baird 1983). The best-known *Acacia* gum is from *A. senegal* --- gum arabic; it, even today, still enjoys a big market in the world although modified starches and cellulose derivatives are replacing some traditional uses of plant gums to an ever -increasing extent. As a result, world demand (all uses) for gum arabic has fallen from 70,000 tons in 1970 to 24,000 tons in 1990-1991.

The genus *Acacia* (Family *Leguminosae*, sub-family *Mimosoideae*) is one of the largest in the Plant Kingdom and now known to comprise ~1100 species and it is the second largest within the Family Fabaceae (Ross 1979). In Australia alone, 729 *Acacia* species were described ten years ago (Maslin and Pedley 1982), the majority of the remaining species being indigenous to Africa and America. The *Acacia* genus still provides many complex botanical problems of nomenclature and classification, one of which is the continuous increase in the number of *Acacia* species identified, making previous classifications outdated and incomplete, also the continuous changes and increases in the names and "fine structure" of sub-species and varieties (Anderson 1978a).

The most useful botanical classification from a chemical point of view is still that of Bentham (Bentham 1875) who divided *Acacia* into 6 Series and 15 sub-series. Although various revisions have been necessary, Bentham's divisions, based on habit, inflorescence and geographical distribution, are Series 1, PHYLLODINEAE, contains 570 spp., subdivided into 8 sub-series; Series 2, BOTRYOCEPHALAE, 32 spp.; Series 3, PULCHELLAE, 14 spp.; Series 4, GUMMIFERAE, 60 spp. with 3 sub-series; Series 5, VULGARES, 75 spp. with 4 sub-series; Series 6, FILICINAE, 2 spp.. Species from Series 1 are native to Australia, Hawaii and New Caledonia; Series 2 and 3 are native to Australia; Series 4 and 5 are found throughout tropical and semi-tropical parts of the world; and Series 6 are native to South America. Chemical analyses of more than 120 different *Acacia* species gum exudates from Series 1, 2, 4 and 5 have now been carried out (Anderson 1978a; Anderson and Dea

1969b), with the conclusion that each *Acacia* species exudes a gum that is characteristic of that species regardless of where it is grown geographically, the chemical composition and physical properties of the exudate from each *Acacia* species differing, often very considerably, from that of other species.

This Chapter presents an analytical and structural study of gum exudates from 8 *Acacia* species (including *A. senegal* for comparative purposes) from different sources belonging to Series 4, GUMMIFERAE and Series 5, VULGARES together with their amino acid composition which is one of the factors essential for a more complete understanding of their properties, biosynthesis and tertiary structures. Furthermore, their ^{13}C NMR spectra have been studied in order to elucidate the major structural differences. This is of special importance for certain *Acacia* gums such as *A. seyal* (gum tahla) and *A. sieberana* , which are frequently used as adulterants in commercial blending with gum arabic because they can be bought in African markets at about 25% of the price for food grade gum arabic from *Acacia senegal.*

5.2 Origin of Gum Samples

Belonging to Series 4 *Gummiferae*:
- *Acacia leucospira* Brenan gum from Somalia.
- *Acacia seyal* gum from Sadore, Niger.
- *Acacia sieberana* gums from Sadore, Niger; and West Africa[a].
- *Acacia paolii* Chiov. gum from Kenya.

Belonging to Series 5 *Vulgares*:
- *Acacia thomasii* gum from Somalia.
- *Acacia sp. nov.*(F.& S.85) gum from Somalia.
- *Acacia senegal* gum from Sadore, Niger.
- *Acacia cheilanthifolia* Chiov. gum from Somalia.

All those gums were completely soluble in water.

5.3 Results and Discussion

Gums of the *Gummiferae* series are characterized mainly by highly positive specific rotation, high molecular weight, and low proportions of rhamnose; whilst the main distinguishing features of gums from the *Vulgares* species are significant negative specific rotations, intermediate molecular weight (of the order of $5x10^5$) and the presence of significant proportions of rhamnose (Anderson 1978a). The data in

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[a]: The analytical data of *A. sieberana* gum from West Africa are not showed in this Thesis; its ^{13}C NMR spectrum (Fig. 5.2) presented only for comparative purposes for different locations.

Table 5.1 show that *A. senegal* gum from Niger is similar to that from Sudan except for its slightly higher intrinsic viscosity (19 ml/g).

Table 5.1 Analytical data for *Acacia* gum samples

	Gum samples							
	A. leucos.	*A. seyal*	*A. sieber.*	*A. paolii*	*A. thomas.*	*A. sp.nov.*	*A. senegal*	*A. cheila.*
H_2O%	13.7	11.7	10.3	13.8	10.0	13.8	12.3	13.7
Ash%	5.79	2.2	0.7	1.2	n.d.	4.8	2.9	1.7
N%	1.20	0.13	0.40	0.05	0.56	0.47	0.32	0.35
NCF	6.65	6.59	6.24	6.79	6.80	6.55	6.56	6.43
Protein%	8.0	0.9	2.5	0.3	3.8	3.1	2.1	2.3
Methoxyl%	1.78	0.51	0.14	0.3	0.15	0.4	0.18	0.65
$[\alpha]_D$	+31°	+62°	+114°	+90°	-24°	-8°	-32°	-40°
$[\eta]$ ml/g	25	14	13	4	9	37	19	1
E.Wt[a]	640	1690	2200	2300	1430	1110	1200	1580
UAA	28	10	8	8	12	16	15	11
Sugar composition after hydrolysis%								
4-O-MGUA[b]	10	3	1	2	1	2.5	1	4
GUA	18	7	7	6	11	13.5	14	7
Gal	48	36	27	25	60	44	41	45
Ara	24	53	65	64	20	36	31	30
Rha	tr	1	tr	3	8	14	13	10
Amino acid composition (per 1000 residues)								
Ala	50	42	41	61	48	22	28	46
Arg	27	20	59	9	17	8	10	16
Asp	103	65	71	94	110	37	49	49
Cys	21	0	0	0	10	0	7	0
Glu	55	73	63	44	50	30	38	40
Gly	77	52	48	51	49	52	62	42
His	26	40	18	28	17	52	50	47
Hyp	143	236	228	245	302	341	287	288
Ile	40	16	24	29	14	12	10	16
Leu	65	62	49	65	35	63	72	88
Lys	32	23	56	21	41	24	25	46
Met	5	3	3	0	3	0	1	3
Phe	57	43	47	25	16	25	32	16
Pro	70	56	73	59	73	67	63	86
Ser	82	154	78	112	105	157	136	111
Thr	51	55	58	57	40	80	77	63
Tyr	17	17	24	13	27	5	18	11
Val	78	43	60	88	43	25	35	32
NCF	6.65	6.59	6.24	6.79	6.80	6.55	6.56	6.43

a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).

A. seyal and *A. sieberana* gums, the major sources of commercial gum tahla, contain very high proportions of arabinose and very low rhamnose contents with relatively low values of acidity. These gums from Niger show the typical *Gummiferae* series characteristics, with much more arabinose than galactose; the ratios of arabinose:galactose in *A. seyal* and *A. sieberana* gums from Niger are 60:40 and 71:29, respectively, with extremely low rhamnose contents. These data agree well with previous reports for those gum species from different regions (Anderson et al. 1973; Anderson 1978a; Anderson et al. 1984). The specific rotation established for *A. seyal* gum varies from +50° to +65° with low rhamnose contents (0 to 4%) and a nitrogen content (ca. 0.11 ~ 0.19) which is always distinguishably lower than that of *A. senegal* gum. The specific rotation of *A. sieberana* gum has been recorded from +104° to +120°, with a low ash content (<2%) but with 0.3 to 0.4% nitrogen content, a value more similar to that of *A. senegal* gum. *A. seyal* gum from Niger contains ca. 3% 4-O-CH_3 group. Fig. 5.1 shows the ^{13}C NMR spectrum for *A. seyal* gum; Figs. 5.2 and 5.3 show the spectra for *A. sieberana* gums from different locations. *A. paolii* as a member of the Series *Gummiferae* is related to *A. sieberana*, as shown not only from the analytical data but also by its ^{13}C NMR spectrum (Fig. 5.4) which is similar to that for *A. sieberana* gum (Figs. 5.2 and 5.3). The very low nitrogen content in *A. paolii* gum, and the different amino acid compositions, specially a lower Arg content in *A. paolii* gum than in *A. sieberana* gum (9:59), are the main differences between these two species.

A. thomasii gum contains very high proportions of galactose and has an unusual rather simple linear (1→6) β-D-Gal-based structure indicated by its ^{13}C NMR spectrum (Fig. 5.5). But the small sample available may already have been degraded slightly in nature because free arabinose and galactose, but not free rhamnose, are indicated by its NMR spectrum. It also contains a high amount of Asp (ca.11%) in its amino acid composition. *A. sp. nov.* and *A. cheilanthifolia* gums, identified by their collectors Fagg and Styles as members of the *A. senegal* complex, are laevorotatory as is characteristic of *Acacia spp.* of Bentham's series *Vulgares*; they have some analytical features comparable to, but different from, *A. senegal* (Anderson et al. 1983a), e.g. the methoxyl content and intrinsic viscosity are extremely low for *A. cheilanthifolia* gum and very high for *A. sp. nov.* gum. The ^{13}C NMR spectra show that *A. sp. nov.* gum (Fig. 5.6) is very close to *A. senegal* gum except that slightly more C_5 of Araf and C_6 of Gal are involved in linkages than those of *A. senegal* (L.) Willd. gum; and that *A. cheilanthifolia* gum (Fig. 5.7) contains a considerable amount of (1→3) β-L-Araf (ca. 6%, with two types of linkages at 62.9

Acacia seyal gum

ppm	Intensity
176.44	0.8
175.20	1.3
109.20	6.4
107.21	1.8
104.08	1.9
102.98	5.6
102.47	5.6
100.50	1.2
99.69	13.6
97.50	11.0
97.12	5.3
83.87	5.9
81.79	25.0
80.45	9.3
79.51	10.5
78.85	4.2
76.67	6.5
76.34	12.8
75.93	11.8
74.86	7.7
73.95	10.3
73.66	15.5
72.98	12.1
72.56	18.4
71.76	13.7
69.61	10.0
68.55	19.5
66.94	9.7
65.58	1.4
65.29	1.3
63.96	12.4
62.17	13.4
61.73	11.0
61.16	22.8
59.86	6.7

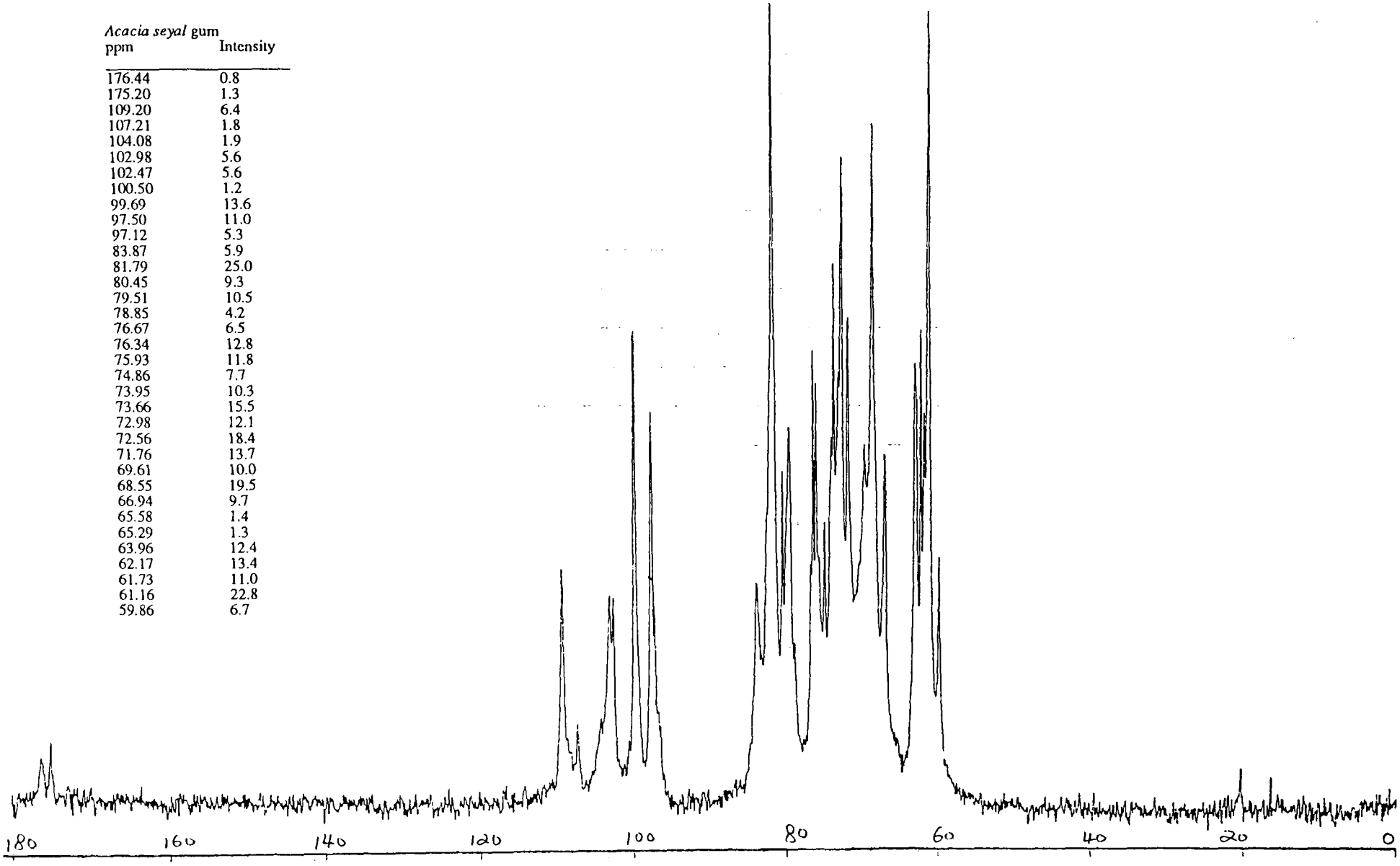

Fig. 5.1 ^{13}C NMR spectrum for *Acacia seyal* gum from Niger

Acacia sieberana gum

ppm	Integral	Intensity
108.55	0.9	2.0
108.32	1.4	2.6
103.31	0.5	2.2
103.15	0.5	2.6
102.86	1.2	3.1
102.71	0.6	2.8
102.51	0.7	2.4
98.92	5.7	7.9
98.47	0.3	2.2
97.93	3.7	4.9
96.91	16.1	14.9
83.26	1.0	2.8
82.39	30.1	27.1
80.99	12.0	10.2
79.90	2.1	6.3
79.79	1.0	6.3
78.99	9.5	13.7
78.55	19.9	18.4
76.68	7.0	10.5
76.27	1.1	3.7
75.61	1.7	3.7
75.42	1.0	3.6
74.93	3.2	5.3
74.41	7.2	12.3
73.46	9.4	13.8
73.05	17.8	21.1
72.34	1.9	6.8
71.97	5.4	10.2
71.74	1.7	7.3
71.52	1.8	7.7
71.31	1.7	6.4
70.97	1.2	4.7
70.73	1.2	4.4
70.60	1.1	4.8
70.39	1.5	5.0
69.93	1.2	4.9
69.53	2.7	5.5
68.37	1.2	4.3
68.01	1.2	3.1
63.22	24.7	28.7
62.78	7.5	15.2
62.28	5.4	10.7
61.20	11.2	10.6
60.64	1.3	4.0
59.90	1.9	4.0

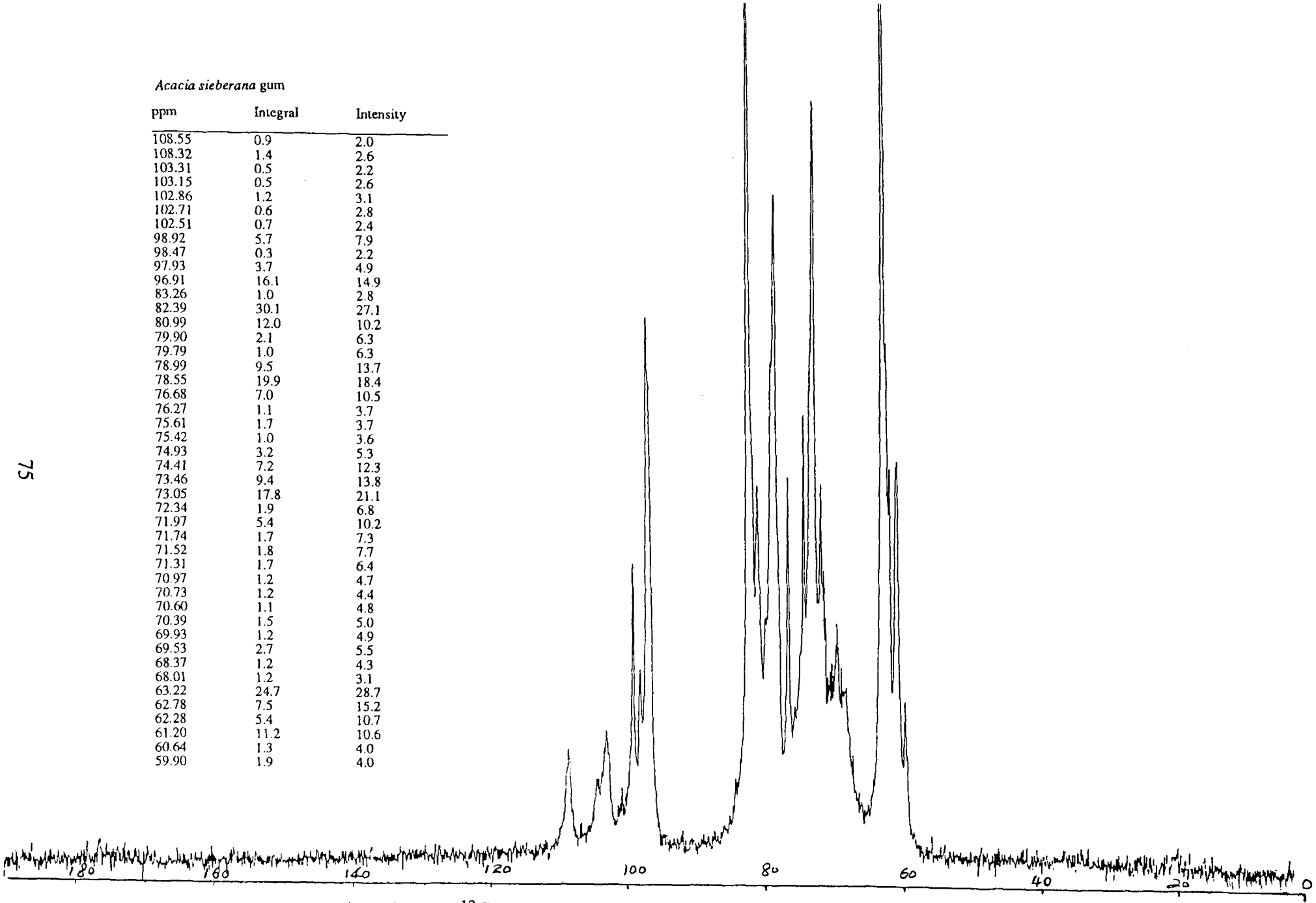

Fig. 5.2 ^{13}C NMR spectrum for *Acacia sieberana* gum from West Africa

A. sieberana gum

ppm	Intensity
108.44	2.5
102.94	2.6
99.02	6.4
97.03	11.0
82.42	20.1
81.10	9.3
78.71	14.9
76.78	8.0
74.50	9.8
73.23	16.9
70.00	5.7
63.34	25.0
61.21	10.1
20.50	1.3

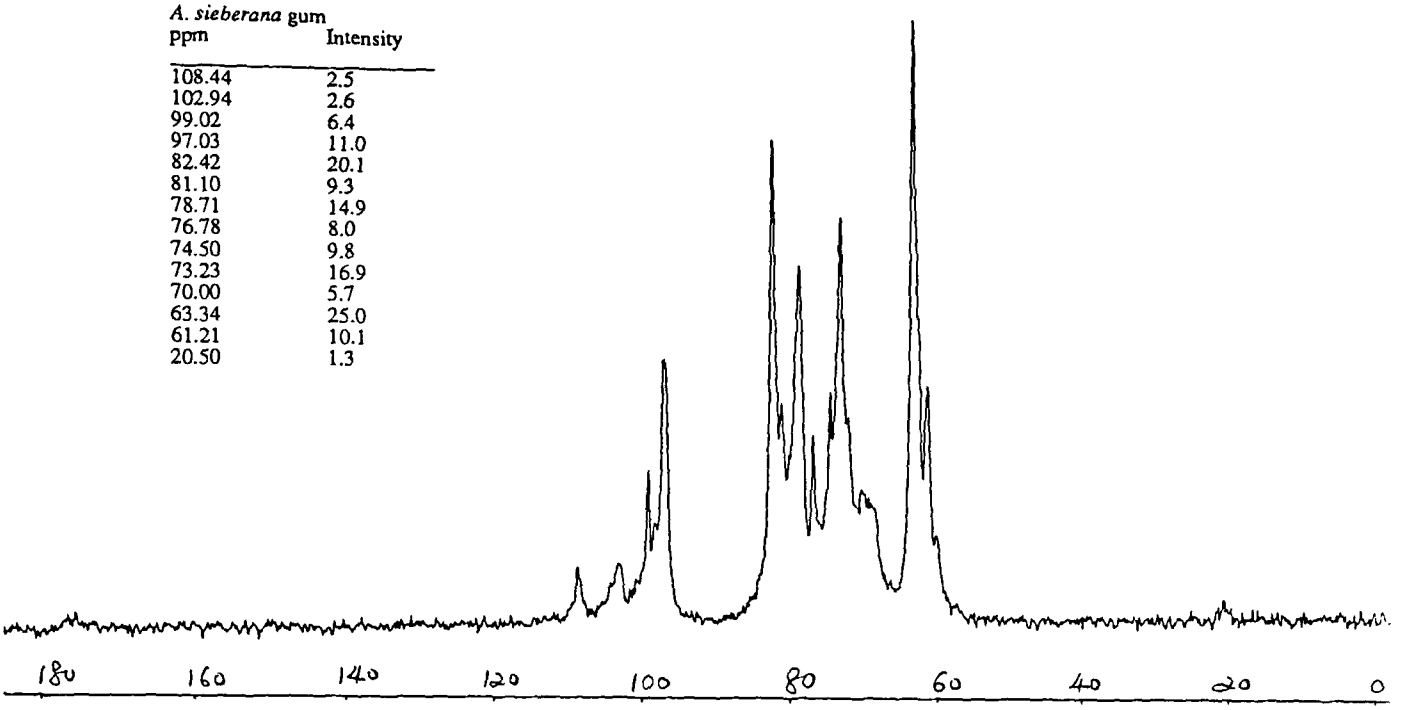

Fig. 5.3 ^{13}C NMR spectrum for *Acacia sieberana* gum from Niger

A. paolii gum

ppm	Intensity	ppm	Intensity
108.84	5.5	72.01	8.7
103.03	4.3	71.89	8.8
99.11	8.3	71.73	8.9
98.14	5.4	71.45	7.4
97.04	14.7	70.80	6.1
84.45	3.9	70.52	7.0
83.78	3.1	70.03	9.0
82.59	25.7	69.17	6.5
81.78	12.9	68.89	6.3
81.44	11.6	68.56	5.1
79.42	15.7	68.42	5.0
78.67	19.4	68.33	5.0
76.78	12.5	63.42	23.4
76.10	4.5	62.90	12.5
75.90	4.3	62.41	10.2
74.63	13.3	61.27	14.9
74.24	9.1	60.30	3.1
73.23	22.3	60.12	3.3
72.44	7.5		

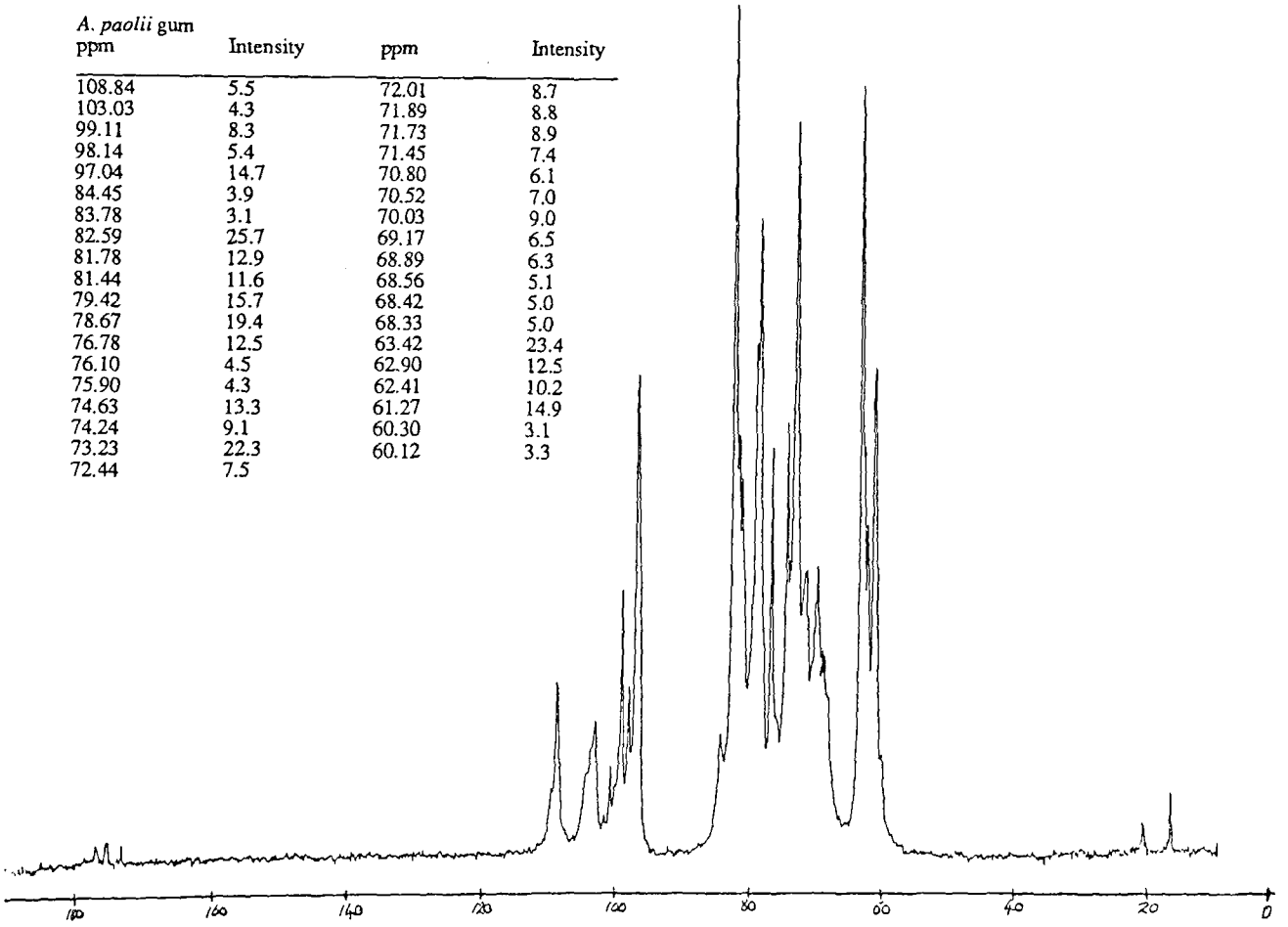

Fig. 5.4 ^{13}C NMR spectrum for *Acacia paolii* gum from Kenya

A. thomasii gum

ppm	Intensity	ppm	Intensity
103.35	12.1	69.97	8.9
102.55	5.4	69.27	12.4
100.67	5.6	68.88	19.7
96.68	1.3	66.32	2.0
96.37	3.9	60.96	5.2
92.31	1.4	16.45	1.9
81.28	1.7		
79.03	5.0		
76.11	3.1		
75.45	1.7		
75.10	5.6		
74.20	5.7		
73.70	15.5		
73.24	7.2		
72.55	16.6		
71.85	7.3		
70.68	18.3		
70.22	6.6		

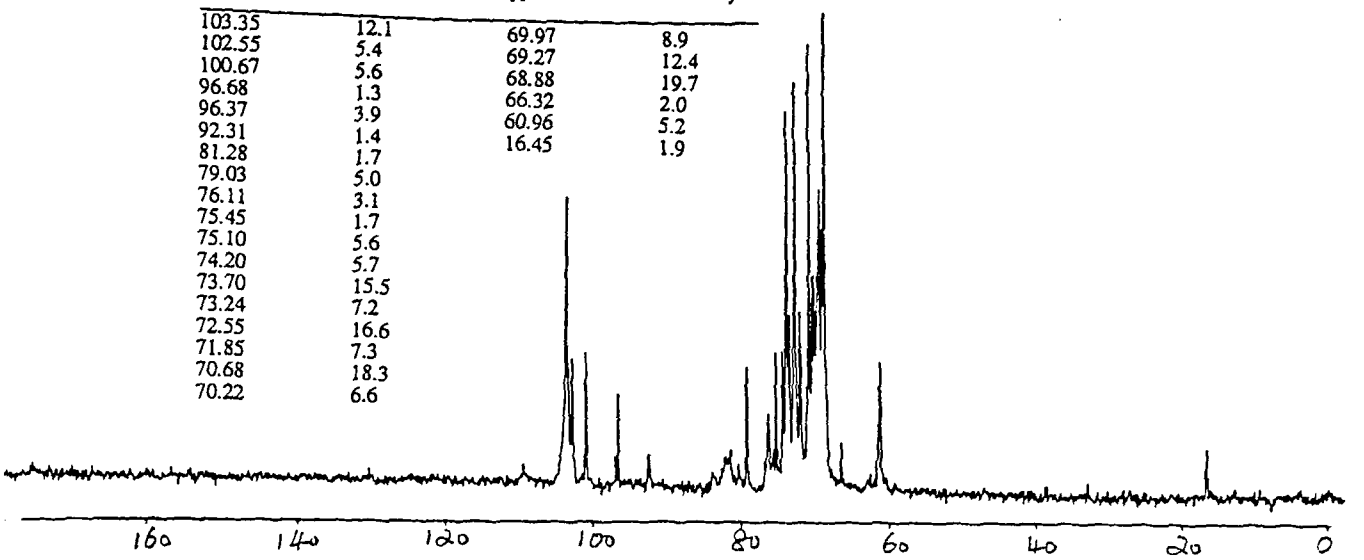

Fig. 5.5 ^{13}C NMR spectrum for *Acacia thomasii* gum δ(ppm)

A. sp. nov. gum

ppm	Intensity
175.12	4.0
109.42	5.0
108.07	3.5
103.62	5.8
102.90	8.7
100.58	14.7
100.00	6.1
99.23	2.0
84.83	3.1
83.78	3.8
82.79	4.6
81.32	5.0
80.63	5.5
80.09	7.5
79.63	5.3
79.00	12.0
76.70	4.2
76.03	9.2
74.84	3.1
74.64	2.2
74.17	12.7
73.32	18.4
72.39	4.3
71.90	16.4
71.33	10.3
70.90	5.8
70.23	19.5
69.99	25.0
69.23	20.5
68.90	22.9
68.48	11.3
63.21	1.4
61.21	22.6
16.50	10.6

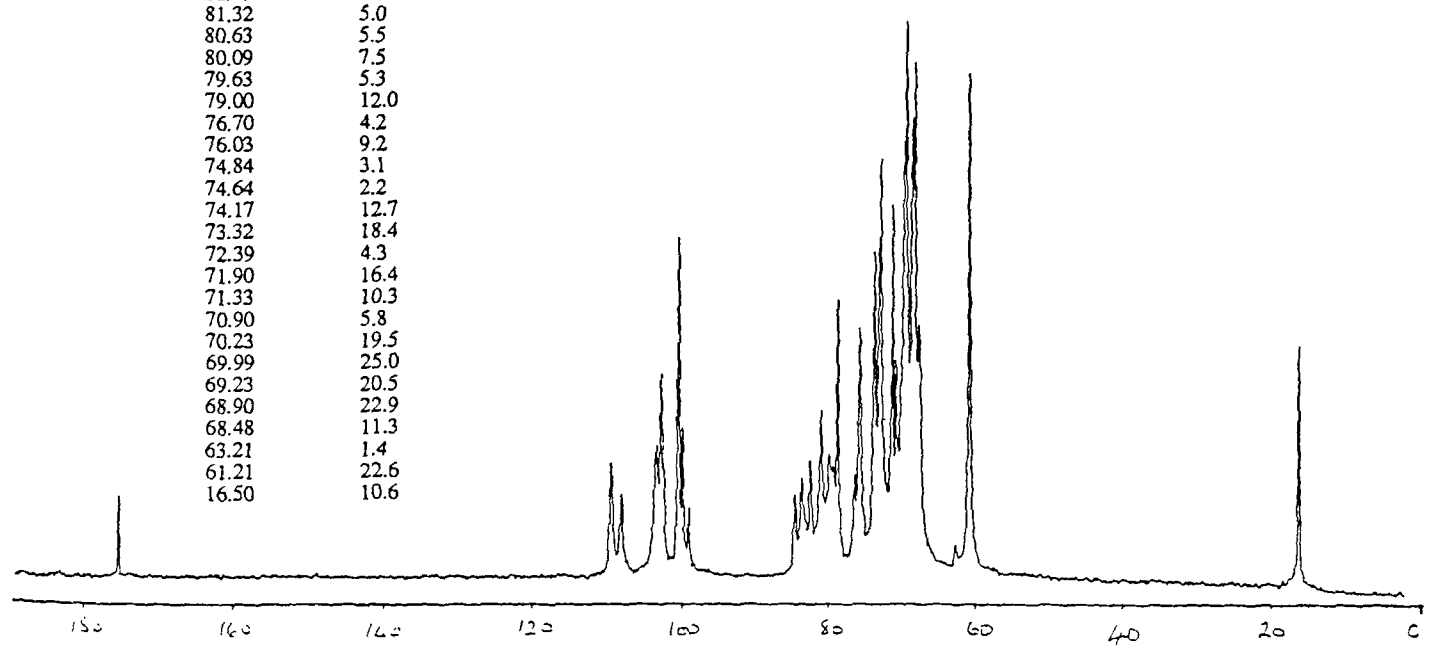

Fig. 5.6 ^{13}C NMR spectrum for *Acacia sp. nov.* gum δ(ppm)

ppm and 63.2 ppm, and C_1 at 101.6 ppm). These are much higher than in *A. senegal* gum. The spectrum also shows structurally that it possesses more mobile side- chains than *A. senegal* gum because of the sharp resonances.

A. leucospira gum is dextrorotatory and indeed comparable to *A. seyal* type of gum in having a comparatively high methoxyl and negligible rhamnose content; however, there are structural differences between them because *A. leucospira* gum has comparatively high nitrogen and uronic acid contents and high viscosity. Its ^{13}C NMR spectrum (Fig. 5.8) reveals that the gum indeed contains very high 4-O-CH_3 (59.8 ppm) and an unusual acetyl content (20.6 ppm, and C=O at 173.2 ppm), in addition, this gum spectrum shows (1→2) β-L-Araf (C_5 at 62.5 ppm and C_1 at 99.6 ppm), and β-L-Arap residues involved in at least 4 different sorts of linkage (99.2, 98.2, 97.2 and 96.8 ppm respectively). These arabinose linkages occur in both *A. seyal* gum and *A. sieberana* gum, but there is only a very small amount of α-L-Araf (107 to 109 ppm) in *A. leucospira* gum comparable to *A. seyal* gum and *A. sieberana* gum. Furthermore, this gum is low in Hyp (14.3%) and Ser (8.2%), but high in Asp (10.3%), Gly (7.7%) and Phe (5.7%) contents and it has a high ratio of Ile/Leu (40:65).

Present studies and previous reports have revealed that *Acacia* gums from the *Gummiferae* Series have some distinctive different features from those in the *Vulgares* Series. The latter have negative rotations, and contain considerable amount of rhamnose (>8%) and an Ile:Leu ratio between ca. 1/3 ~ 2/3. The gums from *Gummiferae* species have positive rotations, negligible amounts of rhamnose (<4%), and the Ile:Leu ratio is generally very low (<1/3). In general, the *Gummiferae* Series gums contain high proportions of arabinose, and also the following fragments

4-O-Me-β-D-GlupA (1→6) β-D-Gal (1→
and α-D-GlupA (1→4) D-Gal (1→

have been reported (Anderson and Dea 1969b, Anderson and Cree 1968b). Sequential Smith-degradations have shown that some *Gummiferae* gums e.g. *A. arabica* (Anderson et al. 1967) and *A. nubica* (Anderson and Cree 1968a) gums possess highly ramified inner structures composed of (1→3) and (1→6) linked D-Gal residues in different proportions, arabinose-containing side chains, joined mostly to C_3 of otherwise (1→6) linked galactose units. In addition, there is evidence that fragments of L-Araf (1→2)-L-Araf(1→ are unusually prominent in this type of polysaccharide (Stephen 1983), in fact it is α-L-Araf(1→2)β-L-Araf(1→, as indicated by the ^{13}C NMR spectra. After partial hydrolysis,

A. cheilanthifolia gum

ppm	Intensity	ppm	Intensity
175.20	1.9	74.84	3.7
109.84	4.5	74.28	15.6
109.49	12.6	74.02	14.8
108.26	9.6	73.72	19.3
103.40	18.9	73.32	20.4
102.61	6.4	72.61	16.1
101.56	5.9	71.95	12.1
100.72	11.1	70.75	19.6
100.13	3.7	70.31	24.3
99.39	2.2	70.05	21.1
83.90	11.5	69.80	15.7
83.69	13.5	68.95	23.4
82.82	5.1	68.64	22.3
81.90	9.2	68.36	11.5
81.35	20.2	63.29	5.5
80.29	10.2	62.94	7.1
79.11	9.2	61.32	24.5
76.77	12.7	61.19	24.3
76.33	10.9	16.54	5.8
75.86	11.0		

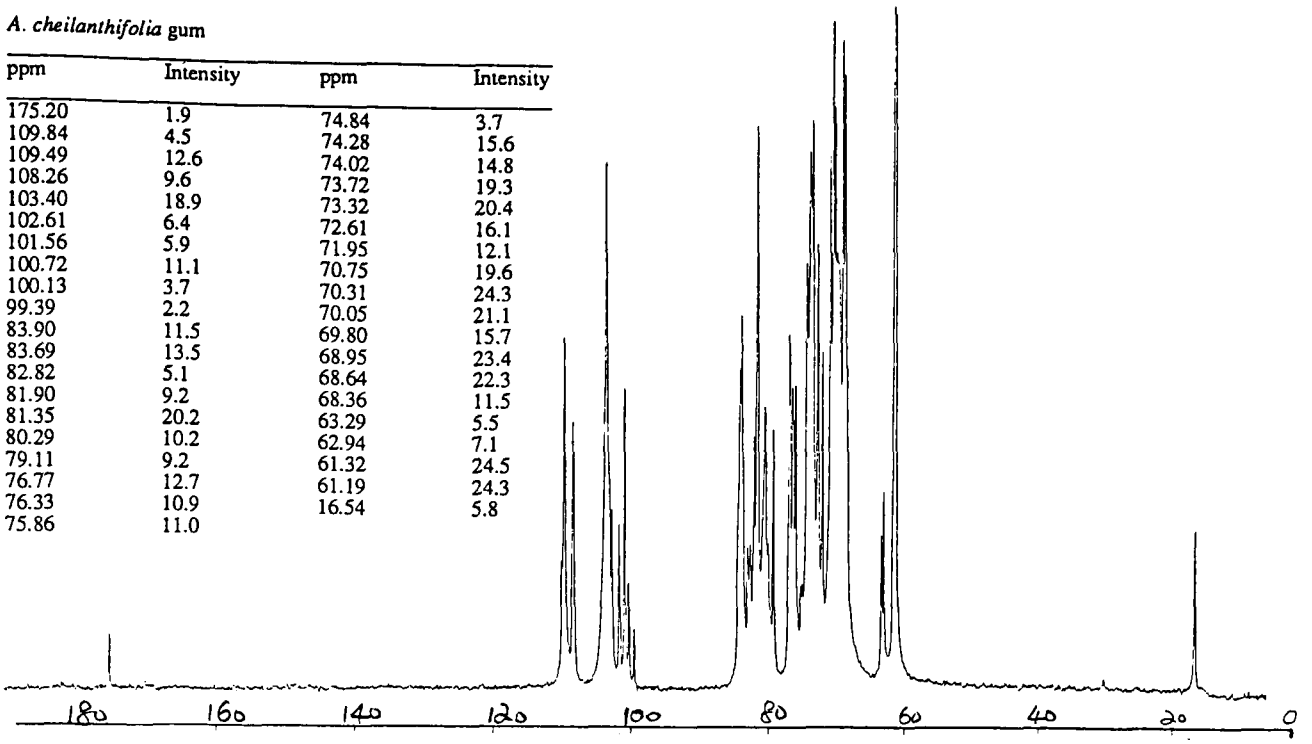

Fig. 5.7 ^{13}C NMR spectrum for *Acacia cheilanthifolia* Chiov. gum δ(ppm)

A. leucospira gum

ppm	Intensity	ppm	Intensity
173.80	2.2	71.62	20.7
173.69	1.9	70.85	21.7
173.21	2.2	70.28	22.3
104.35	12.1	69.97	25.2
103.76	9.6	69.37	13.3
103.50	9.2	68.94	19.7
103.17	10.2	68.70	22.9
100.75	6.9	67.43	2.7
100.11	2.7	67.12	2.5
99.56	8.22	66.40	2.9
99.19	4.3	66.20	1.8
98.19	2.3	63.93	2.0
97.15	4.9	63.54	7.7
96.80	4.9	63.37	8.3
83.51	2.0	63.01	5.4
82.48	13.8	62.53	4.8
82.06	11.6	62.17	2.4
81.38	5.3	61.16	13.6
81.06	4.0	60.06	14.9
80.22	4.8	59.85	15.3
79.34	5.9	20.59	9.9
78.75	5.9		
78.30	3.8		
76.82	13.3		
76.03	6.7		
75.18	25.1		
74.65	10.1		
74.32	13.1		
73.25	18.1		
72.68	22.8		
72.09	20.9		
71.88	21.4		

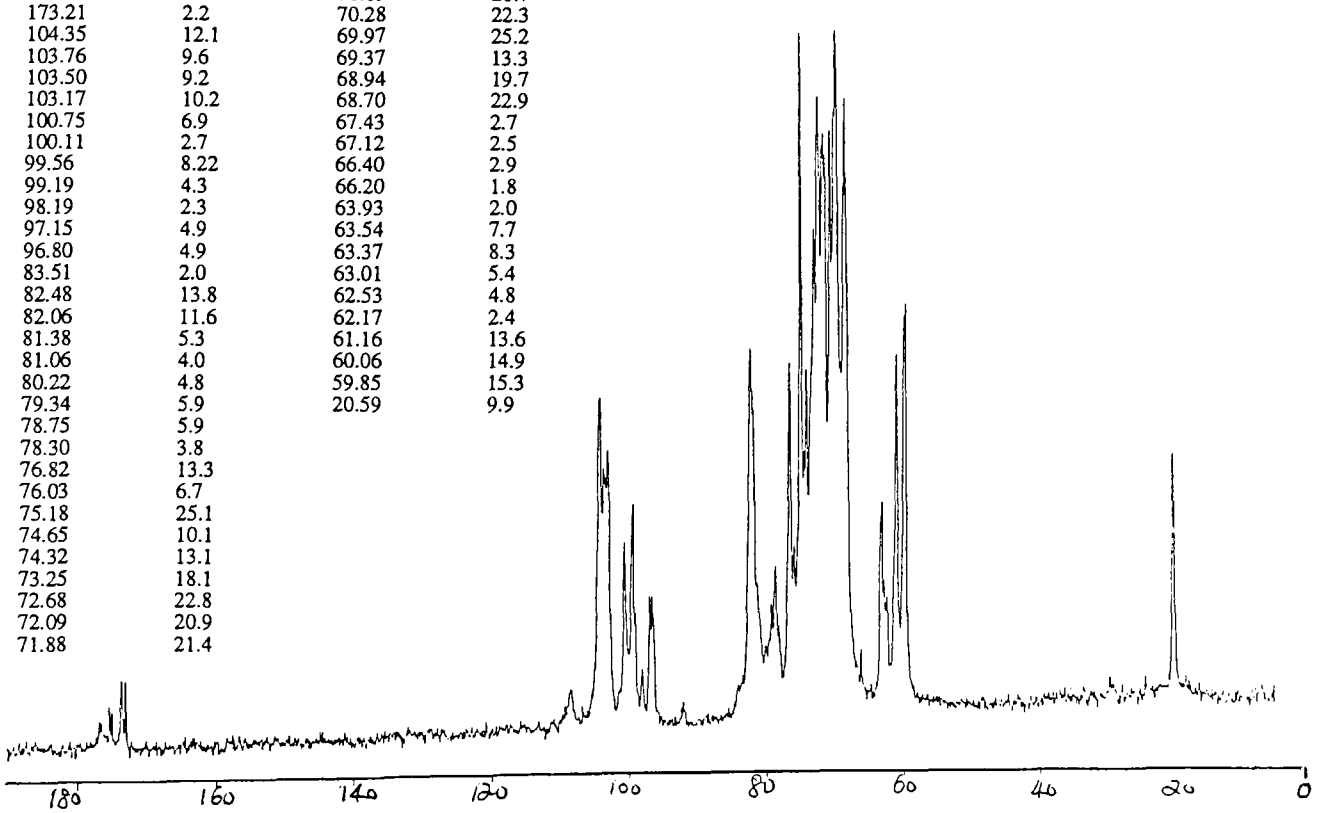

Fig. 5.8 ^{13}C NMR spectrum for *Acacia leucospira* Brenan gum δ(ppm)

β-L-Araf(1→3)-L-Araf(1→,
β-L-Arap(1→3)-L-Araf(1→,

linkages are present as well (Stephen 1983). In contrast, the *Vulgares* Series gums normally contain a considerable amount of α-L-Rham (8~18%). A possible structure for *A. senegal* gum (Street and Anderson 1983) illustrates the great complexity of the *Vulgares* gums. There is little possibility that the exact nature of the branched side-chains or the sequence of their attachment to the (1→3) and (1→6) linked D-galactan core structure can be determined precisely (there are always small structural differences between any two samples) although a repeating structure of some kind may also be involved as the gum's synthesis may result from an enzymatic-controlled process. But terminal fragments of 4-O-Me-β-D-Gal (1→, and α-L-Rham (1→4)-D-GlupA (1→6) β-D-Gal (1→, were earier established in *A. senegal* gum by the base degradation of methylated gum (Aspinall and Young 1965; Aspinall and Rosell 1977). The well-established mixed linkage 1→3 and 1→6 β-D-galactan core of *A. senegal* gum is also present in other *Vulgares* species e.g. *A. laeta* and *A. campylacantha* (=*polyacantha*) gums (Anderson and Munro 1970; Anderson and Dea 1971).

By ^{13}C NMR spectra, such structural differences and possible linkages can be recognised for each gum. The core linkages can also be shown by spectra for a range of degraded gums. The structural differences between *A. seyal*, *A. sieberana* (*Gummiferae*) and *A. thomasii* (*Vulgares*) gums are described below.

5.3.1 Structural Studies of Acacia seyal Gum by NMR

A. seyal (Del.) gum ("gum tahla") is not a food-permitted gum but it is quite often commercially misrepresented as "gum arabic" or blended with *A. senegal* gum to increase profitability. From the chemical point of view, the O-Methyl sugars identified by examination of methanolysis and hydrolysis products from methylated *A. seyal* gum (Anderson et al. 1968b) allowed a possible structure to be produced. The rhamnose and glucuronic acid would be completely eliminated from that structure after a first Smith-degradation, and only a β-D-galactan could remain after a 4th Smith-degradation (Anderson and Yin 1988). Table 5.2(a) shows the data for the anomeric regions of *A. seyal* gum from a ^{13}C NMR spectrum, the constituent sugars and their configurations have been already listed in Chapter 3. Table 5.2(b) shows all the assignments possible from the spectrum and Table 5.2(c) presents the possible structural interpretations from the spectrum. Resonances in non-anomeric regions are frequently overlapped because of the peak broadening caused by less mobile similar

carbons.

The main differences in structural features between *A. seyal* and *A. senegal* gums are that *A. seyal* gum contains much less internal (1→3) linked α-L-Araf (107.2 ppm) and α-L-Rham (100.5 ppm and C_6 at 16.5 ppm), but much more internal (1→2) linked β-L-Araf (99.7 ppm), (1→2) linked β-L-Arap (97.5 ppm) and a higher content of 4-Meβ-GlupA than *A. senegal* gum, which has a high content of (1→3) linked α-L-Araf, but only very little of β-L-Arap in its structure.

Table 5.2(a) The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum for *A. seyal* gum (gum tahla)

Chemical shift (ppm)	Intensity	C_1 of
109.2	6.4	α-L-Araf(1→
(108.3)		→α-L-Araf(1→
107.2	1.8	→α-L-Araf(1→Ara(1→
104.1	1.9	α-L-Arap(1→
103.0	5.6	→β-D-Gal(1→ and β-D-Gal(1→
102.5	5.6	→β-D-GlupA(1→ and β-D-GlupA(1→
100.5	1.2	α-L-rham(1→
99.7	13.4	→2)β-L-Araf(1→
97.5	11.0	→ 2)β-L-Arap(1→
97.1	5.3	→2,3)β-L-Arap(1→

Table 5.2(b) ^{13}C NMR chemical shifts(ppm) for *A. seyal* gum and the possible interpretation of monosaccharide constituents

Linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-Rham-(1→	100.5	(70.2	70.0	71.9	68.3	16.5)
→4)β-D-GlupA-(1→	102.5	73.7	74.0	78.9	75.9	175.2
→3,6)β-D-Gal-(1→	103.0	71.8	81.8	69.6	73.0	69.6
→3,4,6)β-D-Gal-(1→	103.0	71.8	81.8	74.0	73.0	69.6
β-D-Gal-(1→	103.0	71.8	73.3	68.5	74.9	61.2
→3)β-D-Gal-(1→	103.0	71.8	81.8	68.5	74.9	61.2
→4)β-D-Gal-(1→	103.0	71.8	73.7	74.9	74.0	61.2
→6)β-D-Gal-(1→	103.0	71.8	72.6	68.5	73.7	69.6
α-L-Araf-(1→	109.2	80.4 79.5	76.7 76.3	83.9	61.2	
→3)α-L-Araf-(1→	108.3 107.2	81.8	83.9	83.3	61.2	
→2)β-L-Araf-(1→	99.7	83.9	73.0	81.8	63.0	
α-L-Arap-(1→	104.1	71.8	72.6	68.5	66.9	
→2)β-L-Arap-(1→	97.5	81.8	72.6	71.8	61.7	
→2,3)β-L-Arap-(1→	97.1	83.3	78.9	73.0	62.2	

Table 5.2(c) ^{13}C NMR assignments for *A. seyal* gum and the possible structural interpretations

δ(ppm)	Intensity	Carbon
59.9	6.7	-OCH_3 in 4-O-Me-β-D-GlupA-(1→
61.2	22.8	α-L-Araf, C_5 and 1,3,α-L-Araf, C_5
		1,& 1,3,& 1,4,β-D-Gal, C_6
61.7	11.0	1,2,β-L-Arap, C_5
62.2	13.4	1,2,3,β-L-Arap, C_5
63.0	12.4	1,2,β-L-Araf,C_5
66.9	9.7	α-L-Arap, C_5
68.5	19.5	α-L-Arap, C_4
		1,&1,3,& 1,6,β-D-Gal, C_5
69.6	10.0	1,3,6,β-D-Gal, C_3
		1,6,& 1,3,6,& 1,3,4,6,β-D-Gal, C_6
71.8	13.7	α-L-Arap, C_2 and 1,2,β-L-Arap, C_4
		β-D-Gal, C_1 (all inter. & term.)
72.6	18.4	1,2,β-L-Arap, C_3 and α-L-Arap, C_3
		1,6,β-D-Gal, C_3
73.0	12.1	1,2,β-L-Araf,C_3
		1,2,3,β-L-Arap, C_4
		1,3,6,& 1,3,4,6,β-D-Gal, C_5
73.7	15.5	1,(4),β-D-GlupA, C_2
		1,&1,4,β-D-Gal, C_3 and 1,6,β-D-Gal, C_5
74.0	10.3	1,(4),β-D-GlupA, C_3
		1,3,4,6,β-D-Gal, C_4 and 1,4,β-D-Gal, C_5
74.9	7.7	1,4,β-D-Gal, C_4 and 1,& 1,3,β-D-Gal, C_5
75.9	11.8	1,(4),β-D-GlupA, C_5
76.3	12.8	α-L-Araf, C_3
76.7	6.5	α-L-Araf, C_3
78.9	4.2	1,2,3,β-L-Arap, C_3
		1,4,β-D-GlupA, C_4
79.5	10.5	α-L-Araf, C_2
80.4	9.3	α-L-Araf, C_2
81.8	25.1	1,2,β-L-Araf,C_4 and 1,3,α-L-Araf, C_2
		1,2,β-L-Arap, C_2
		1,3,& 1,3,6,& 1,3,4,6,β-D-Gal, C_3
83.3	3.8	1,3,α-L-Araf, C_4
		1,2,3,β-L-Arap, C_2
83.9	5.9	α-L-Araf, C_4 and 1,3,α-L-Araf, C_3
		1,2,β-L-Araf, C_2
97.1	5.3	1,2,3,β-L-Arap, C_1
97.5	11.0	1,2,β-L-Arap, C_1
99.7	13.4	1,2,β-L-Araf,C_1
100.5	1.2	α-L-rham, C_1
102.5	5.6	1,(4),β-D-GlupA, C_1
103.0	5.6	β-D-Gal, C_1 (all inter. & term.)
104.1	1.9	α-L-Arap, C_1
107.2	1.8	1,3,α-L-Araf, C_1
(108.3)		1,3,α-L-Araf, C_1
109.2	6.4	α-L-Araf,C_1
175.2	1.3	1,(4),β-D-GlupA, C_6

5.3.2 Structural Studies of Acacia sieberana Gum by NMR

Acacia sieberana D.C. (formerly *sieberiana*) gum is frequently offered in West African commercial gum markets, and belongs to the *Gummiferae* Series. Its analytical data have been well established and are typical of species in this Series (Anderson et al. 1973; Anderson et al. 1983a; Anderson and Wang 1991). In particular, *Acacia sieberana* gum has a highly positive optical rotation (+105° ~ +120°) and contains more than twice as much as arabinose than galactose with a negligible amount of rhamnose. Its nitrogen content is similar to that of *Acacia senegal*. Typical *Acacia sieberana* gum ^{13}C NMR spectra are shown in Figs. 5.2 and 5.3; five major peaks indicating extremely high amounts of arabinose are present.

Table 5.3(a) The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum for *A. sieberana* gum

Chemical shift (ppm)	Intensity	C_1 of
108.5 -108.3	2.0-2.6	α-L-Araf(1→β-L-Arap1→
103.3 -102.9	2.2-3.1	→β-D-Gal(1→ and β-D-Gal(1→
102.5 -102.7	2.4-2.8	→β-D-GlupA(1→ and β-D-GlupA(1→
98.9	7.9	β-L-Arap(1→Ara
97.9	4.9	→2)β-L-Arap(1→
96.9	15.0	→2,3)β-L-Arap(1→ (with 63.2 ppm from C_5)

Table 5.3(b) ^{13}C NMR chemical shifts(ppm) for *A. sieberana* gum and the possible interpretation of monosaccharide constituents

linkage	C_1	C_2	C_3	C_4	C_5	C_6
→4)β-D-GlupA-(1→	102.6	73.5	74.4	79.0	75.6	(175)
β-L-Arap-(1→	98.9	71.0	72.0	72.0	62.8	
→2,3)β-L-Arap-(1→	96.9	82.4	78.6	73.1	63.2	
→2)β-L-Arap-(1→	79.9	81.0	72.4	71.7	62.3	
α-L-Araf-(1→	108.3[a]	79.9	76.7	83.3	60.6	
β-D-Gal-(1→	102.9	72.0	73.5	69.5	75.6	61.2
→3)β-D-Gal-(1→	102.9	70.7	81.0	69.5	75.6	61.2
→3,4)β-D-Gal-(1→	102.9	70.7	81.0	74.9	75.6	61.2

[a]: this peak still appears to be for the terminal arabinose; the chemical shift upfield of that expected may be caused by the neighbouring linkages because there are no assignments at or above 84.00 ppm to support any other possible linkages involved in α-L-Araf(1→.

Structurally, the five major assignments (around 96.9, 82.4, 78.6, 73.1, 63.2 ppm all ±0.2 ppm) may represent the carbons in →2,3)β-L-Arap-(1→ fragments. Another feature is the absence of a 109.3 ppm assignment; only the 108.5 ppm assignment appears in the spectrum to represent the C_1 of terminal α-L-Araf. So β-L-Arap is the dominant sugar unit in this gum. In addition to the 4-O-Me-β-D-GlupA → D-galactose linkage fragment, the

α-L-Araf(1→2 or 2,3 -β-L-Arap(1→....→ Gal
and β-L-Arap(1→2 or 2,3 -β-L-Arap(1→....→ Gal

linkages may also be the terminal groups in the structure. But the major terminal sugar units are β-L-Arap rather than α-L-Araf in this gum. Fig. 5.9 is a ^{13}C NMR spectrum for acid hydrolysed *A. sieberana* gum; by comparing it with Fig. 5.10, which is the ^{13}C NMR spectrum for acid hydrolysed *A. seyal* gum, the arabinose content is even higher in *A. sieberana* gum (Fig. 5.9) than in *A. seyal* gum (Fig. 5.10) although both are arabinose-dominated and galactose and arabinose are the only two major sugars, which is well in agreement with the results obtained by Paper Chromatography by the earlier investigators.

5.3.3 Structural Studies of Acacia thomasii Gum by NMR

The ^{13}C NMR spectrum of *Acacia thomasii* gum (Fig. 5.5) shows this gum possesses a surprisingly simple structure. Free galactose and arabinose, but no free rhamnose, were also present in the spectrum: this is very unusual and may have been caused by natural degradation after collection of the gum (through poor storage conditions) or whilst the gum was on the tree (e.g. for up to a year after its original formation). The sugar composition of the gum can be obtained directly by the assignments in the spectrum data shown on Table 5.4(a). Table 5.4(b) shows the possible interpretation of the intersugar constituent peaks. Considering the intensities of the assignments at 60.96, 61.15 ppm (which represent C_6 of free β- and α-D-Gal) and at 96.68, 92.31 ppm (for the C_1), the conclusion can be reached that almost every galactose in the structure is involved in (1→6) linkages, because there are only relatively very small peaks between 79.04~85.00 ppm, which represent (1→3), (1→4) linkages of galactose and the (1→3) linkage of arabinose. *A. thomasii* gum is therefore based on a very high proportion of a (1→6) linked galactan. The free arabinopyranoses detected may have been degraded from Araf terminals because of the traces of 109.0 ppm assignments seen; the free galactose may have been degraded from the galactose terminal or Araf→Gal(1→ terminal units. The proposed structural fragments of this polysaccharide structure therefore appear to be:

A. sieberana gum after acidic hydrolysis

ppm	Intensity
100.71	1.2
96.37	19.4
96.05	7.8
92.20	9.5
91.90	4.1
82.68	1.1
81.02	2.2
80.93	2.4
76.17	1.2
75.84	1.5
75.23	1.3
74.73	6.9
73.80	1.0
73.30	1.2
72.40	7.7
72.07	18.7
71.53	25.2
71.06	2.2
70.87	1.4
70.66	2.2
70.43	1.7
70.07	4.0
68.96	4.7
68.82	5.3
68.38	17.3
68.24	15.3
68.14	25.1
66.04	17.6
62.41	1.4
62.10	9.0
60.89	5.3
60.69	7.0

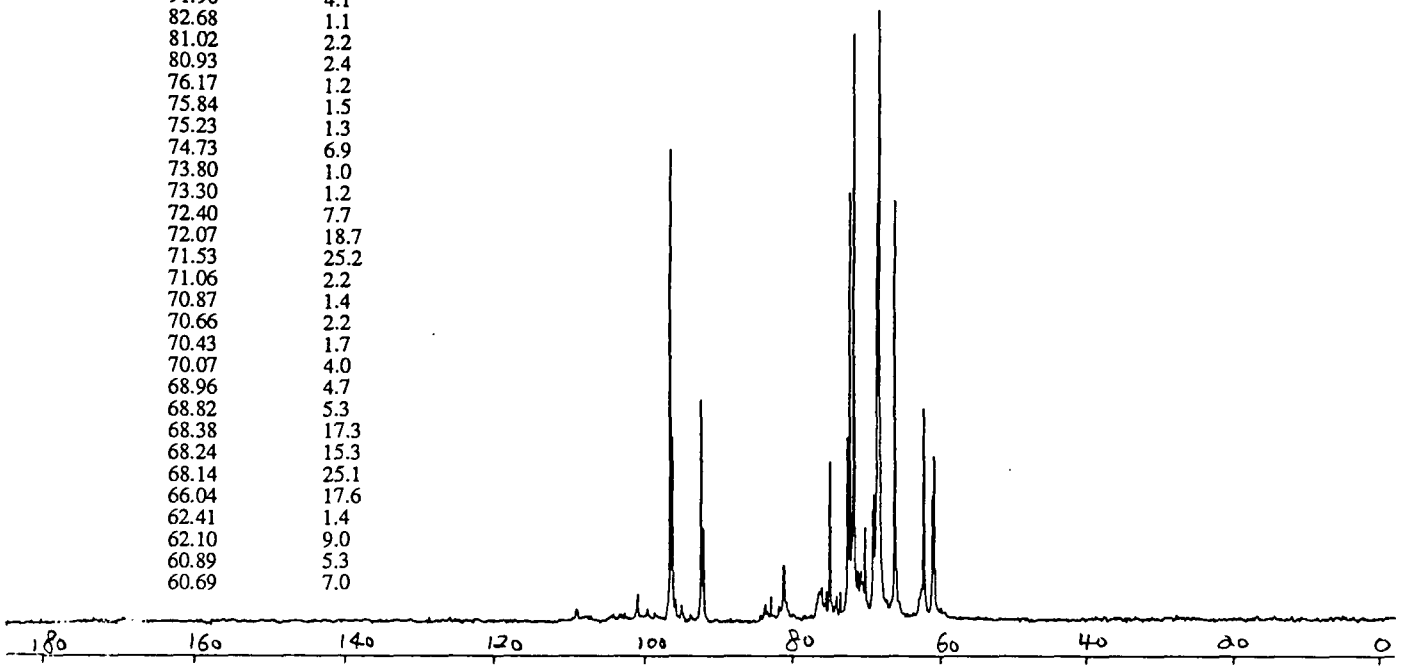

Fig. 5.9 ^{13}C NMR spectrum for *Acacia sieberana* gum after acidic hydrolysis δ(ppm)

A. seyal gum after acidic hydrolysis

ppm	Intensity
100.97	1.2
96.94	15.5
96.33	9.8
95.72	1.1
92.46	7.8
92.18	4.8
81.30	1.2
76.13	1.1
75.51	1.3
75.00	9.8
74.08	1.1
72.68	9.6
72.35	15.9
71.79	25.0
71.27	1.5
70.35	4.7
69.22	5.4
69.09	6.0
68.65	12.7
68.61	12.7
68.39	22.4
68.28	7.7
66.28	12.4
62.37	6.7
61.11	5.1
60.92	8.5
59.80	1.3

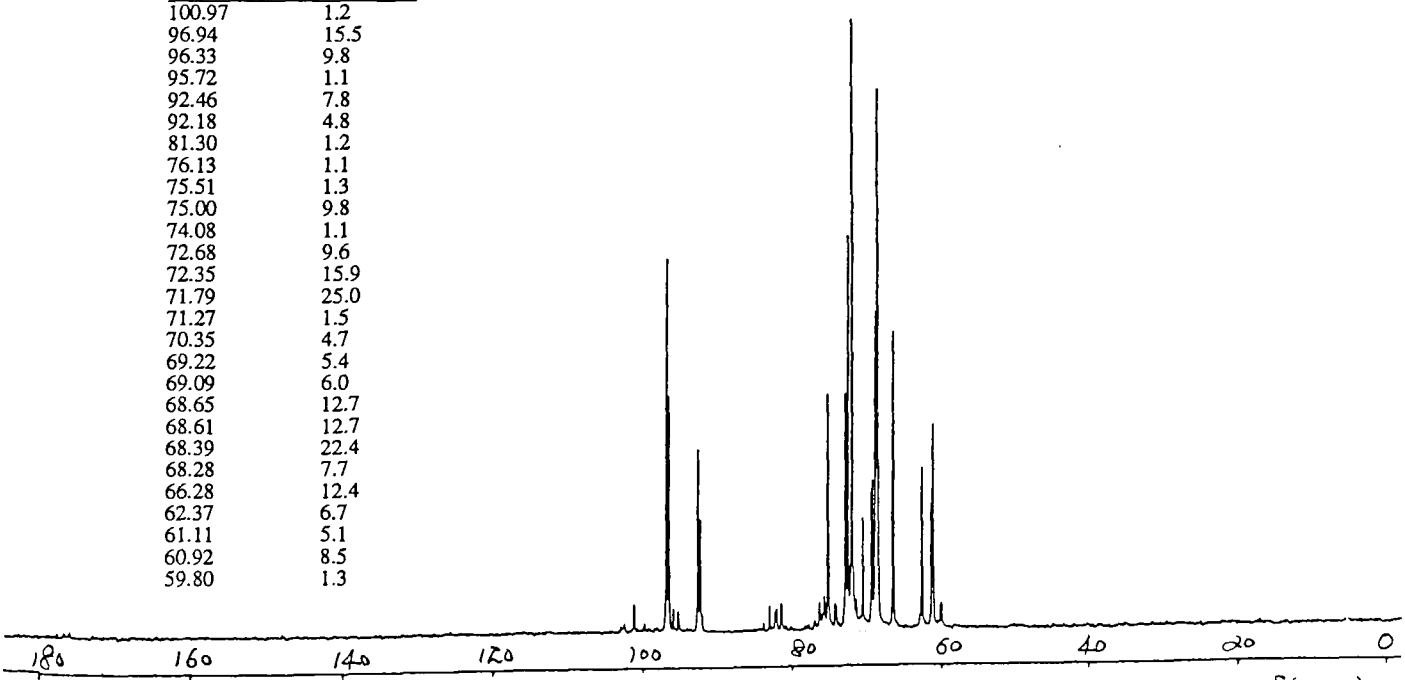

Fig. 5.10 ^{13}C NMR spectrum for *Acacia seyal* gum after acidic hydrolysis δ(ppm)

a large amount of side-chain:
α-L-Araf(1→6)β-D-Gal(1→6)β-D-Gal(1→3,6;4,6;3,4,6)β-D-Gal(1→

terminal chains:
α-L-Rham(1→4)β-D-GlupA(1→6)β-D-Gal(1→6)β-D-Gal(1→

and 4-O-Me-β-D-GlupA(1→6)β-D-Gal(1→...

with the core based on a 1,3,6 linked β-D-Galactan.

Table 5.4(a) The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum for *A. thomasii* gum

Chemical shift (ppm)	intensity	C_1 of
103.4	12.1	→β-D-Gal(1→ and β-D-Gal(1→
102.5	5.4	→β-D-GlupA(1→ and β-D-GlupA(1→
100.5	5.6	α-L-rham(1→
96.7	1.3	free α-L-Arap
96.4	3.9	free β-D-Gal
(92.5)		free β-L-Arap
92.3	1.4	free α-D-Gal

Table 5.4(b) ^{13}C NMR chemical shifts(ppm) for *A. thomasii* gum and the possible interpretation of monosaccharide constituents

linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-rham(1→	100.7	70.2	70.0	71.9	68.6	16.5
→4)β-D-GlupA(1→	102.5	73.2	74.2	79.0	75.4	175.1
→6)β-D-Gal(1→	103.4	70.7	72.6	68.9	73.7	69.3
→3,6)β-D-Gal(1→	103.4	70.2	81.3	68.6	75.1	69.3
→4,6)β-D-Gal(1→	103.4	70.7	72.6	76.1	73.2	69.3
→3,4,6)β-D-Gal(1→	103.4	70.7	81.3	74.2	73.2	69.3
free sugars						
α-L-Arap	96.7	71.9	72.6	68.6	66.3	
β-L-Arap	92.5	68.6	68.6	68.6	62.4	
α-D-Gal	92.3	69.3	68.6	69.3	70.7	61.2
β-D-Gal	96.4	71.9	72.6	68.9	75.1	61.0

Chapter 6
Studies of Highly Proteinaceous Acacia Gum Exudates

6.1 Introduction

Acacia gum exudates are proteinaceous polysaccharides, their protein content ranging from ca. 0.2% to as high as 59% in *Acacia hebeclada* gum (Anderson and Farquhar 1979), but normally less than 5% for most *Acacia* gums. It is very important to investigate the amino acid compositions of those highly proteinaceous gums and their fractions in order to try to unravel the differences in structure and functional properties between *Acacia* gums with high and normal proteinaceous contents. Special attention has been given to gum specimens from members of the Series *Juliflorae*, one of the eight subseries in Bentham's PHYLLODINOUS Series (see Chapter 5). Many of the highly proteinaceous *Acacia* gums belong to the *Juliflorae* sub-series such as *A. torulosa* (N%=7.8); *A. stipuligera* (N%=6.3); *A. difficilis* (N%=8.5); *A. tumida* (N%=6.7); *A. eriopoda* (N%=6.8) (Anderson et al. 1983c). A notable exception is *A. hebeclada* (N%=9.4) gum, which belongs to the *Gummiferae* Series (Anderson and Farquhar 1979). Botanically, the *Juliflorae* is the most complex group of phyllodinous *Acacias* and it has been shown (Anderson and Gill 1975) that the *Juliflorae* comprises a number of species with widely differing chemical properties. These previous studies have revealed that these highly proteinaceous gums normally have high methoxyl contents and very low rhamnose contents and lower molecular weight (Anderson et al. 1983c). In this Chapter, the ^{13}C NMR method has been applied to reveal their structural features and their differences from gum arabic (*Acacia senegal*).

6.2 Origin of Gum Samples

The gums from *Acacia eriopoda* Maiden and Blakely and *Acacia tumida* F.Muell ex Benth. were collected by Mr. T. Willing near Broom, Western Australia, in December 1981 (T.Willing 42 and 41); *Acacia difficilis* Maiden gum was collected by Dr. M. Tindale 40 km south of Pine Creek, Northern Territory, on 10 July 1979 (NSW 108566).

6.3 Analytical and NMR Results

6.3.1 Results

Table 6.1 shows the analytical data for these three highly proteinaceous gums; some of the data are quoted from previous studies (Anderson et al. 1983c). Data for *Acacia senegal* (gum arabic) are included for comparative purposes. The whole gum ^{13}C NMR spectra are shown in Figs. 6.1-6.3 and two sugar (after acid hydrolysis) ^{13}C NMR spectra in Figs. 6.5 and 6.6.

Table 6.1 The analytical data for highly proteinaceous *Acacia* gums

	A.eriopoda	*A.difficilis*	*A.tumida*	*A.senegal*
Ash%	1.7*	1.2*	2.2*	4.1
N%	6.8	8.5	6.7	0.33
NCF	6.85	6.34	6.75	6.60
Protein%	47	54	45	2.2
Methoxyl%	1.3*	4.2*	4.2*	0.26
$[\alpha]_D$	+5°	-10°	-48°	-32°
[η]ml/g	19*	30*	11*	17
E.Wt[a]	1180*	530*	660*	1040
U.A.A.	15	33	27	17
Solubility%	100	100	100	100
Mwx10^5	3.6	3.0	8.3	12
Sugar composition after hydrolysis of whole gum%				
4-O-MGUA[b]	8	25	25	2
GUA	7	8	2	15
Gal	16	31	28	39
Ara	69	36	30	31
Rha	<1	<1	<1	13

a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).
* (Anderson et al. 1983c).

6.3.2 Structural Studies and Discussion

These three highly proteinaceous gums have widely differing specific rotations; +5°, -10° and -48° respectively. Compared with the analytical data for gum arabic, they have low ash contents, high methoxyl contents, more arabinose than galactose, and none of them have more than 1% of rhamnose. *A. eriopoda* gum contains the highest arabinose content recorded (Ara:Gal=81:19) so far for a gum exudate; and Fig. 6.6 shows its ^{13}C NMR spectrum after acid hydrolysis; the very high intensities of the arabinose resonances confirms that the arabinose content is 4 times more than galactose in *A. eriopoda* gum; but the arabinose content shown by the ^{13}C NMR

Acacia difficilis gum

ppm	Integral	Intensity
175.68	0.5	2.4
130.54	2.0	1.9
116.64	1.5	1.5
103.78	5.0	3.1
103.50	2.1	2.9
103.03	1.2	2.9
102.68	11.1	11.2
99.99	16.8	11.7
81.98	37.3	38.5
75.47	1.2	3.7
74.97	14.8	17.1
74.46	16.3	20.0
74.07	7.2	6.9
73.33	5.3	8.3
72.89	17.8	20.4
72.36	2.2	3.4
71.91	8.1	5.0
70.78	2.6	3.0
70.05	8.1	7.6
69.95	2.5	7.4
69.80	1.7	6.9
69.67	12.5	7.3
68.42	9.6	5.6
66.91	2.3	1.9
63.28	26.0	17.9
62.38	2.1	2.7
61.31	1.1	1.9
60.88	3.6	2.9
60.32	5.4	3.6
59.89	7.4	12.2
59.00	7.1	3.6
57.42	11.8	5.7
52.58	6.4	3.7
52.22	2.6	2.8
36.37	2.1	2.0
34.61	11.7	4.5
18.25	1.8	2.1

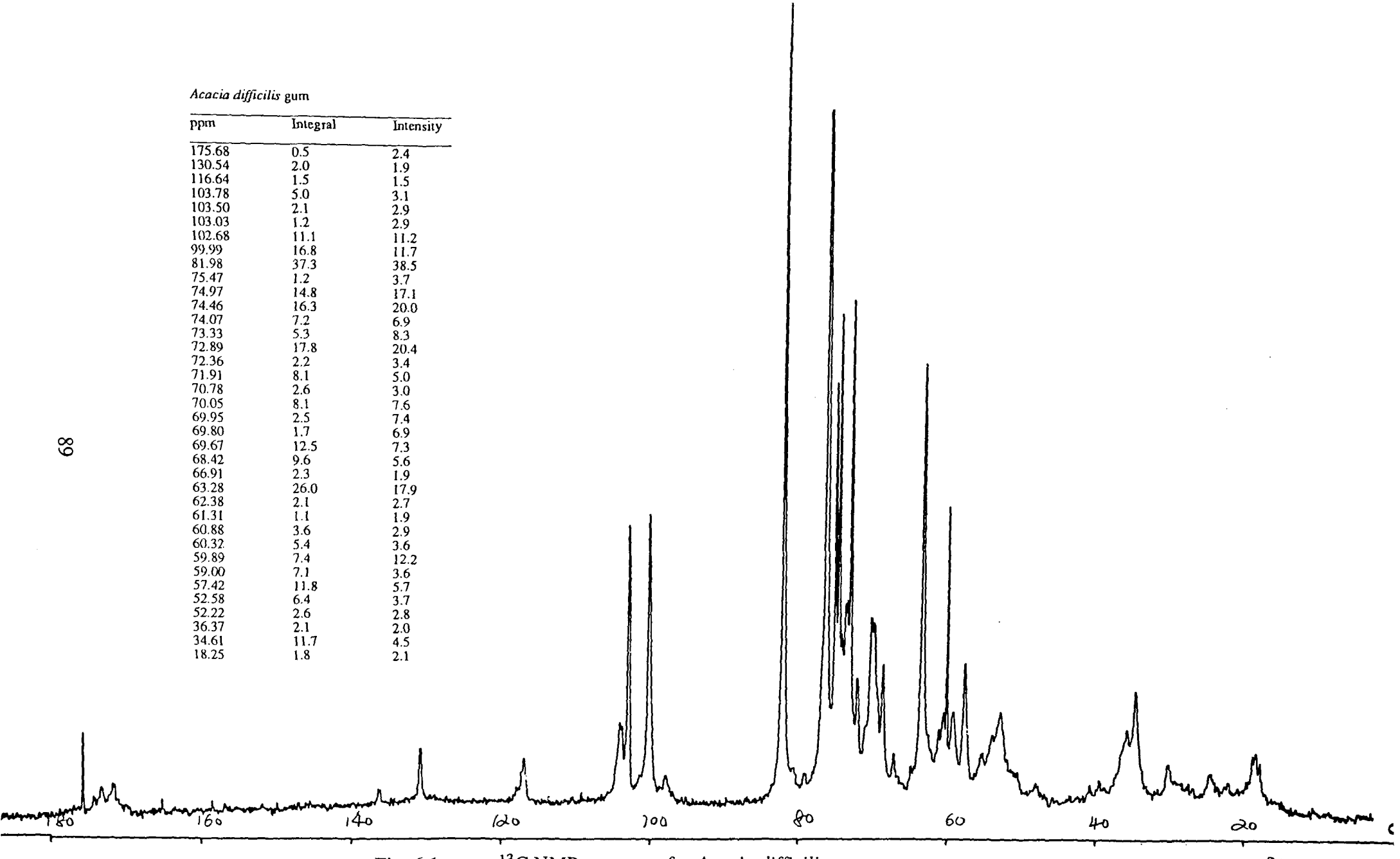

Fig. 6.1 ^{13}C NMR spectrum for *Acacia difficilis* gum

Acacia tumida gum

ppm	Integral	Intensity
175.72	4.0	1.7
175.64	3.7	1.5
173.33	2.9	1.2
173.12	2.7	1.3
130.89	4.5	1.7
115.52	4.5	1.8
104.23	7.2	2.3
103.78	3.2	2.1
103.70	4.7	2.0
102.74	17.9	5.9
102.43	6.2	6.1
101.04	2.9	1.1
100.71	1.1	1.1
100.52	2.0	1.4
100.01	19.2	4.2
97.78	12.2	2.6
97.44	2.1	1.0
96.52	7.3	6.1
95.89	1.5	1.5
92.40	3.3	2.7
83.41	4.8	1.2
82.33	18.7	6.6
82.08	58.8	22.0
77.89	4.2	1.6
76.67	25.4	5.0
76.30	9.1	5.6
76.02	23.6	12.6
75.13	34.0	14.2
74.11	16.4	5.6
73.80	16.3	10.2
73.62	7.9	7.1
73.14	42.0	20.3
72.44	8.6	8.6
72.73	16.9	9.0
71.89	10.9	5.9
69.67	9.6	4.1
69.30	27.4	12.6
68.78	18.8	10.1
66.78	4.1	1.7
63.70	8.4	2.6
63.12	28.8	6.2
62.50	8.0	2.5
61.22	10.5	5.6
61.03	10.2	7.1
60.61	7.8	2.5
60.04	17.7	13.5
59.23	4.9	2.0
57.44	7.9	1.7

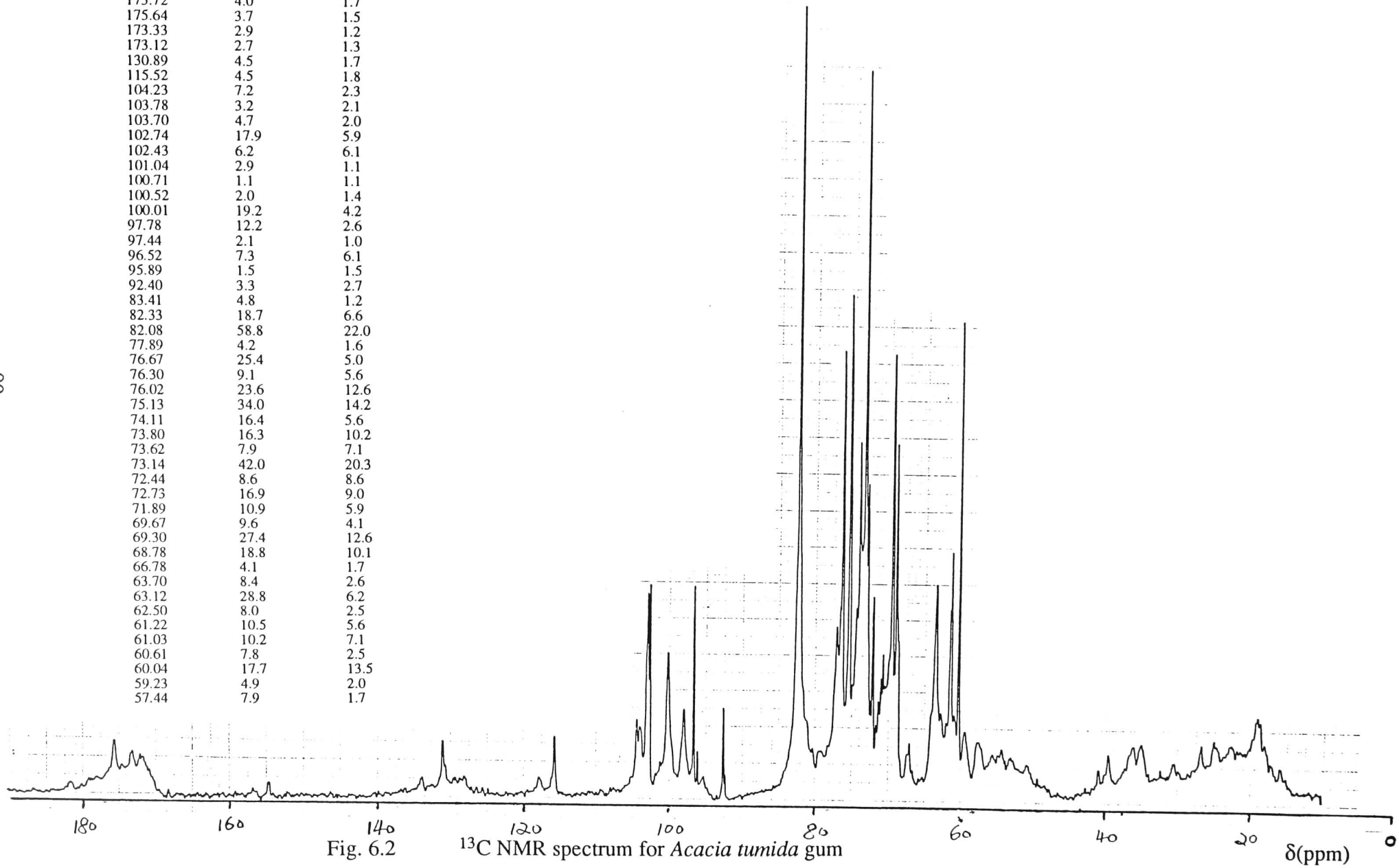

Fig. 6.2 ^{13}C NMR spectrum for *Acacia tumida* gum

A. erriopoda gum

ppm	Intensity
175.60	1.8
130.42	1.4
115.44	1.6
103.60	3.5
102.64	6.4
99.81	7.3
97.74	4.0
82.00	25.1
76.53	11.9
76.01	14.8
75.13	15.2
72.89	23.3
69.78	5.2
68.50	4.6
63.13	13.1
61.12	7.7
60.04	10.1
57.31	1.6
34.72	2.0
18.50	2.3

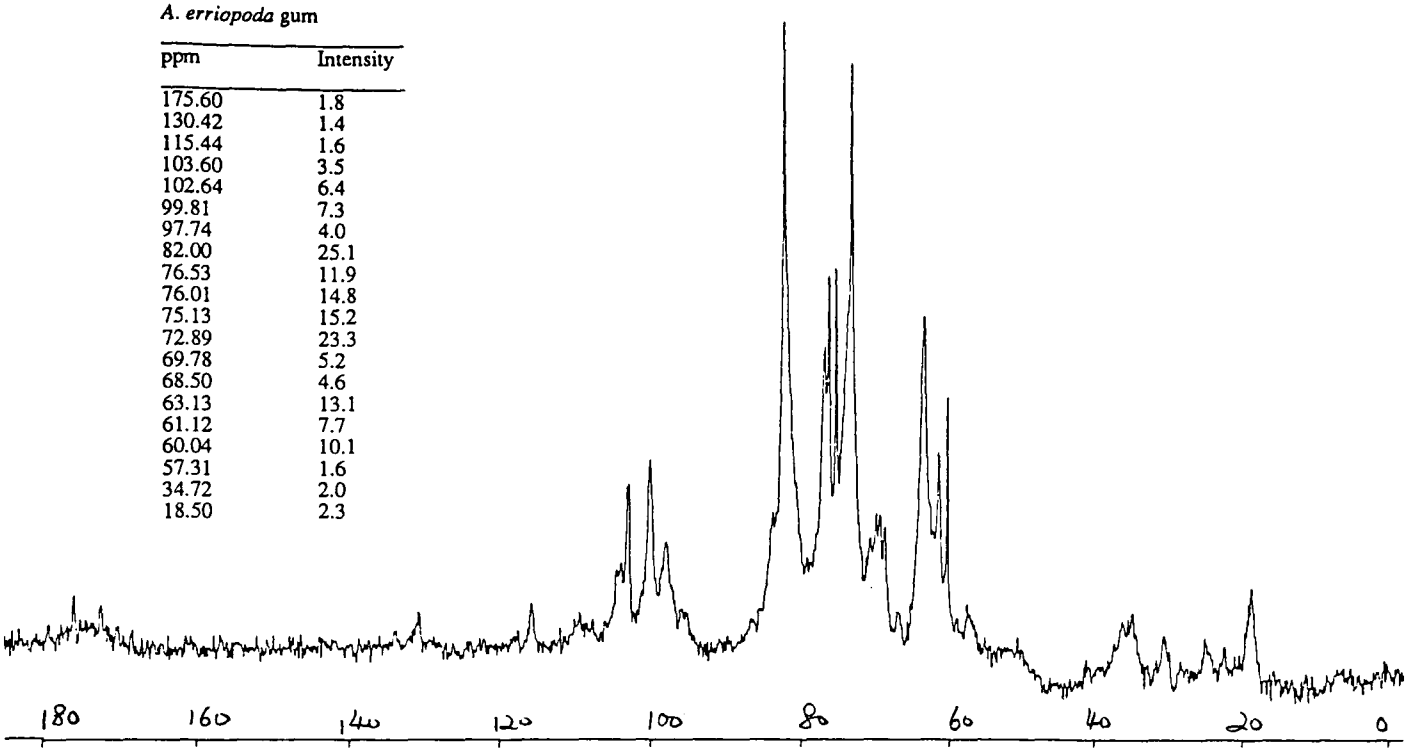

Fig. 6.3 ^{13}C NMR spectrum for *Acacia eriopoda* gum δ(ppm)

A. eriopoda gum (SD1)

ppm	Intensity
173.10	1.6
171.22	1.2
103.89	2.8
103.56	3.0
103.03	6.7
100.12	1.0
98.60	4.5
83.42	5.4
81.78	10.8
79.89	2.8
79.67	2.6
78.70	6.1
76.33	2.2
75.89	2.0
73.21	6.9
67.11	2.3
63.44	5.0
63.23	4.8
62.56	20.0
61.89	4.7
61.56	18.0
61.14	19.1
60.50	8.5
59.12	1.8
57.63	7.8
51.89	1.2
34.45	1.1
18.52	1.5

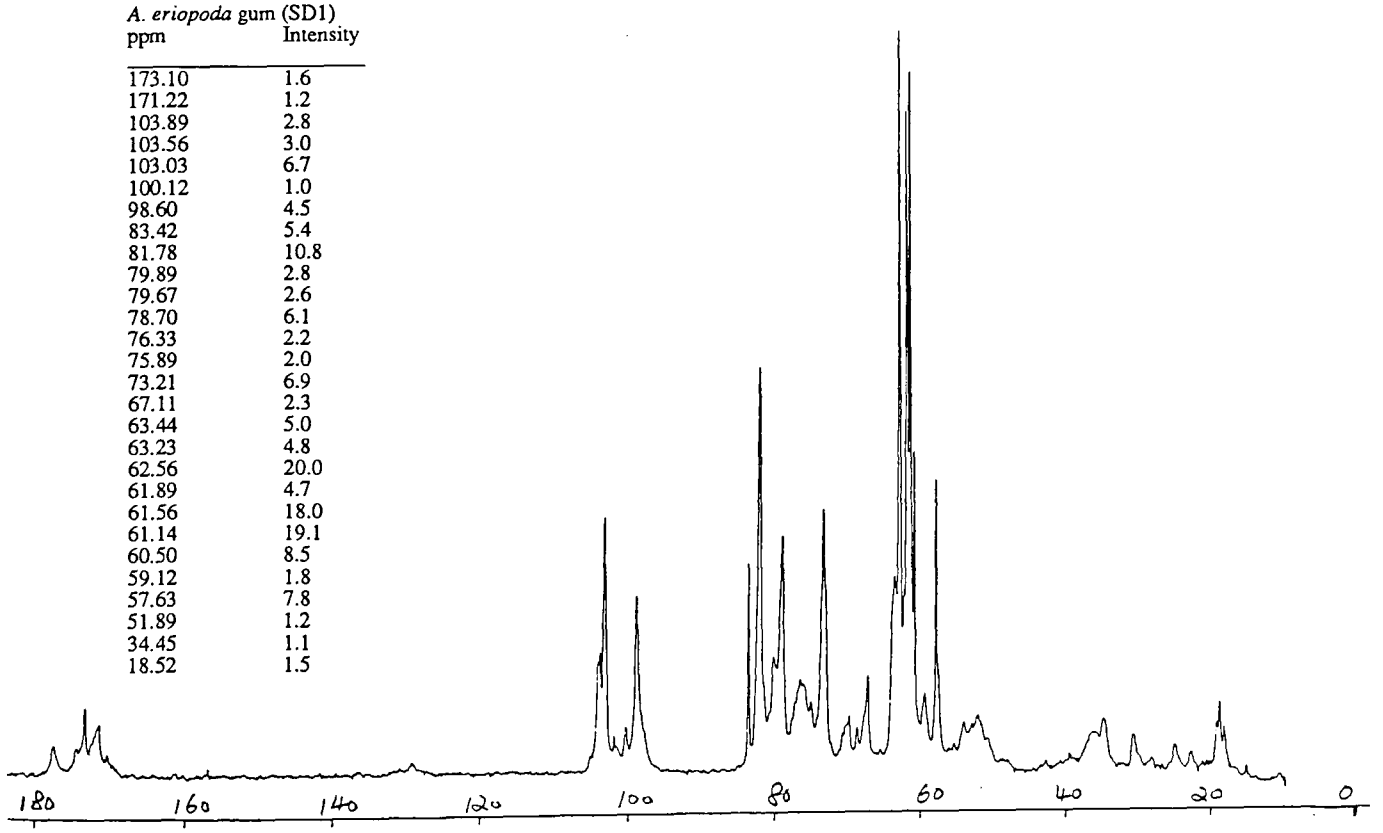

Fig. 6.4 ^{13}C NMR spectrum for degraded *A. eriopoda* gum (SD1) δ(ppm)

A. difficilis gum after acidic hydrolysis

ppm	Intensity	ppm	Intensity
172.70	1.8	68.62	7.4
129.04	2.2	68.41	14.7
128.78	2.5	68.30	10.5
111.54	1.6	68.00	17.1
115.00	2.1	67.78	7.2
101.89	1.8	66.30	1.9
96.20	14.0	65.89	14.8
95.94	11.9	61.91	6.7
95.43	3.6	61.03	4.5
93.50	1.5	60.71	8.1
92.02	6.9	60.50	3.9
91.78	6.1	59.67	5.3
91.64	3.9	58.89	3.4
80.78	5.6	57.78	3.9
80.67	6.4	56.92	3.0
74.30	9.2	54.56	4.4
73.22	5.7	54.13	3.5
72.33	6.5	53.10	2.8
72.23	15.4	48.67	5.1
71.89	14.5	36.30	3.5
71.41	25.5	36.02	3.4
69.56	12.6	35.33	3.7
69.03	5.9	33.14	5.1
		29.41	3.2
		25.78	2.1
		24.30	2.4
		23.89	2.1
		23.30	2.1
		21.56	1.6
		18.21	2.1
		17.78	2.6
		16.89	2.2

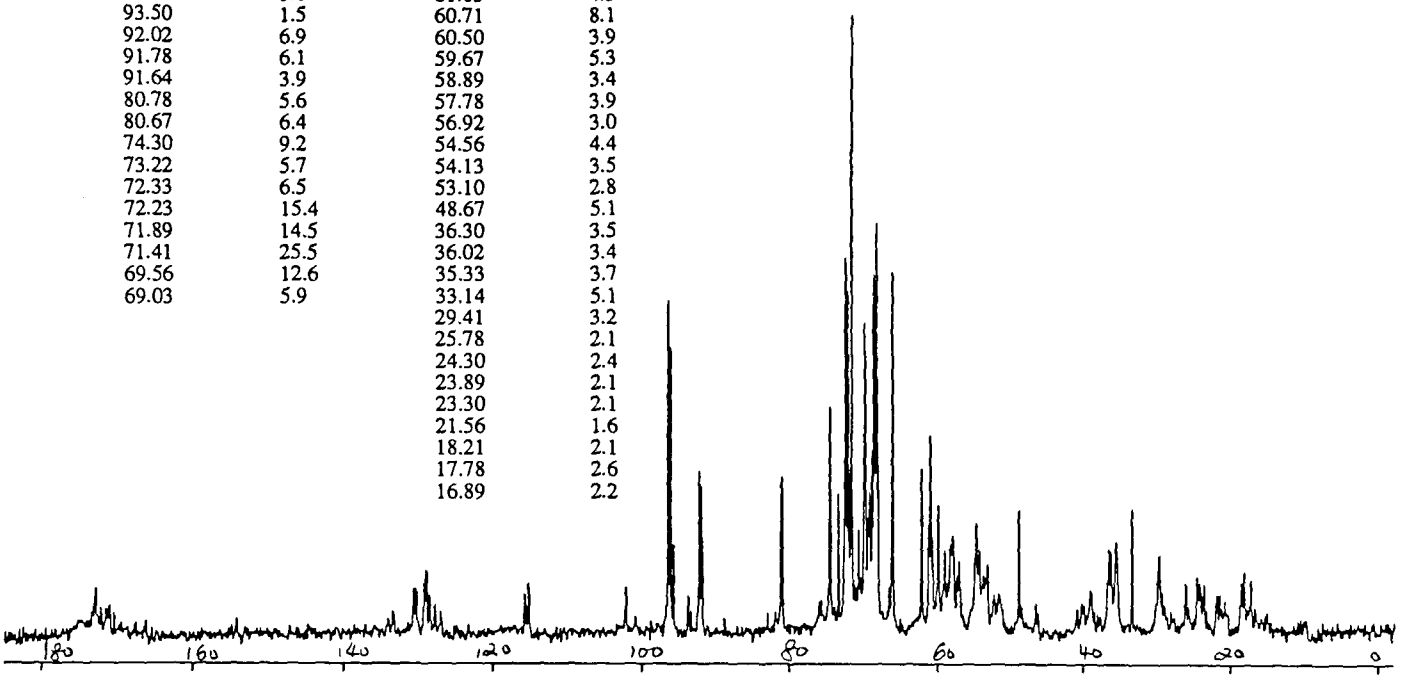

Fig. 6.5 ^{13}C NMR spectrum for *A. difficilis* gum after acidic hydrolysis δ(ppm)

A. eriopoda gum after acidic hydrolysis

ppm	Intensity
96.70	19.3
96.31	4.7
95.72	1.4
92.50	9.5
92.22	2.1
82.01	1.7
75.04	4.4
72.89	2.6
72.67	5.1
72.30	19.2
72.01	2.4
71.67	22.7
70.44	2.5
69.89	4.5
69.21	4.2
69.13	4.0
68.62	14.8
68.54	14.7
68.45	25.1
66.34	19.8
62.31	10.7
61.05	3.6
60.89	5.1
57.30	2.5
54.91	2.0
35.67	2.4

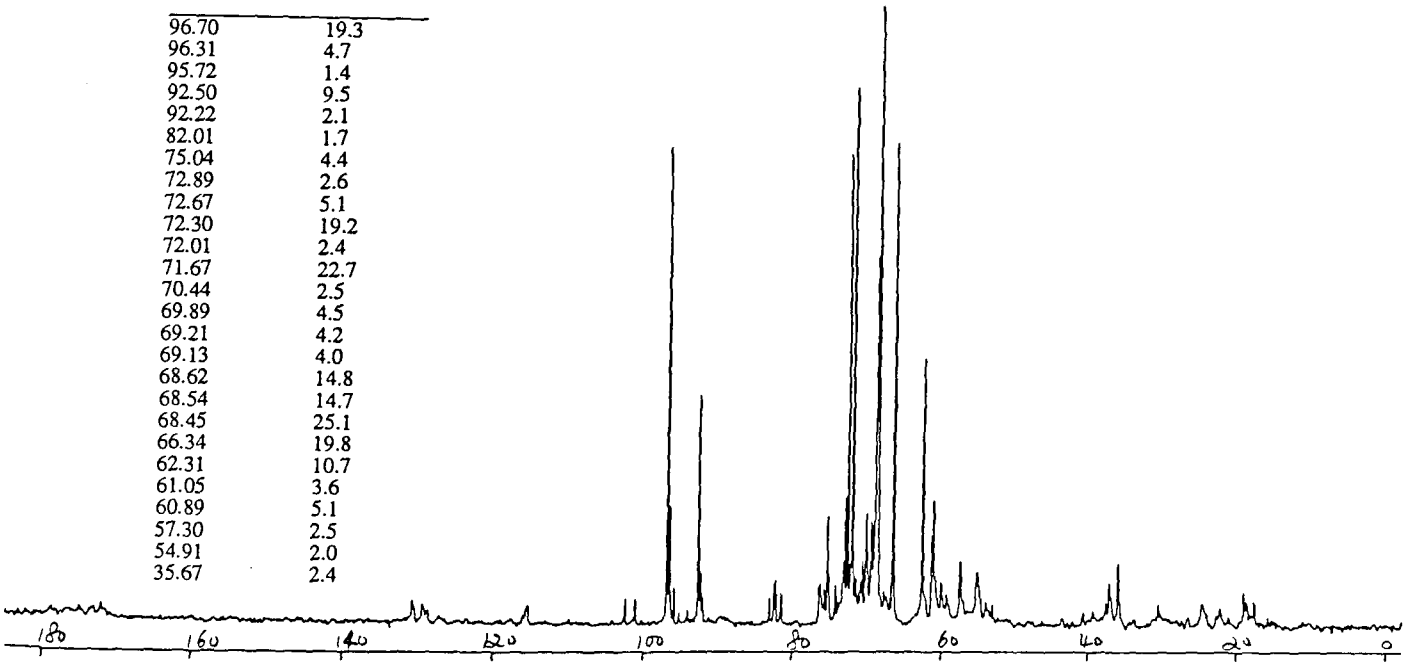

Fig. 6.6 ^{13}C NMR spectrum for *A. eriopoda* gum after acidic hydrolysis δ(ppm)

spectrum (Fig. 6.5) for *A. difficilis* gum is not as high as in *A. eriopoda* gum. *A. difficilis* and *A. tumida* gums contain a very high methoxyl, and high uronic anhydride contents. From their ^{13}C NMR spectra (Figs. 6.1 and 6.2), the possible inter-sugar linkages and their configurations can be deduced as shown in Tables 6.2(a) and 6.2(b). By comparison with the *Acacia senegal* spectrum (Fig. 4.1), distinctive differences from the highly nitrogenous gums can be recognised in terms of the assignments summarised in Chapter 3. References for some polypeptide assignments are quoted below.

Table 6.2(a) The composition of sugar units indicated by some assignments in the ^{13}C NMR spectrum for *Acacia difficilis* gum (Fig. 6.1)

Chemical shift (ppm)	C_1 of
103.8;103.5	→β-D-Gal(1→ and β-D-Gal(1→
102.7	(4-O-Me)→β-D-GlupA(1→ and β-D-GlupA(1→ ? →β-D-Gal(1→
99.9	β-L-Araf(1→β-D-Gal(1→ (with 81.9, 76.2, 72.9, 63.3 ppm from C_4, $_2$, C_3, C_5)
173.1-175	C=O groups
130.5	Aromatic ring carbons (C_{arom}) in polypeptide chain
116.6	Heteroaromatic ring carbons in polypeptides
59.9	O-CH_3 in 4-O-Me-β-D-GlupA

Because the highly proteinaceous gums contain sufficient protein to be detected by NMR methods, information can be obtained from the ^{13}C chemical shifts of the constituent amino acids according to the results of several investigations of amino acids by ^{13}C NMR (Horsley et al. 1970; Dorman and Bovey 1973) which served to establish the following resonances:

(1) Carboxyl groups between 168 - 183 ppm.
(2) α-Carbons 40 -65 ppm.
(3) β-Carbon atoms 17 - 70 ppm.
(4) γ- and δ- Carbons 17 - 50 ppm.
(5) Aromatic and heteroaromatic ring carbons 110 -140 ppm.

Table 6.2(b) The composition of sugar units indicated by some assignments in the ^{13}C NMR spectrum for *Acacia tumida* gum (Fig. 6.2)

Chemical shift (ppm)	C_1 of
104.2	α-L-Arap(1→ (with 67.2 -67.8 ppm from C_5)
103.8;103.7	→β-D-Gal(1→ and β-D-Gal(1→
102.7	→β-D-GlupA(1→ and β-D-GlupA(1→ ?→β-D-Gal(1→
102.4	→β-D-GlupA(1→ and β-D-GlupA(1→
101.0	→3)β-L-Araf(1→ (with 62.9 -63.0 ppm from C_5)
100.7;100.5	α and β-L-Rham(1→
100.0	β-L-Araf(1→β-D-Gal(1→ (with 82.1, 76.0, 73.1, 63.1 ppm from C_4, $_2$, C_3, C_5)
97.4-97.8	→2)β-L-Arap(1→ (with 62.5 ppm from C_5)
95.9	Free β-D-Galp C_1 (61.0 ppm for C_6)
95.9	Free β-D-GlupA C_1
92.37	Free α-D-Galp C_1 (61.2 ppm for C_6)
92.07	Free α-D-GlupA C_1
173.1-175	C=O groups
130.5	Aromatic ring carbons (C_{arom}) in polypeptide chain
116.6	Heteroaromatic ring carbons in polypeptides
60.0	O-CH_3 in 4-O-Me-β-D-GlupA

The features common to the ^{13}C NMR spectra for those highly proteinaceous gums can be summarised as follows. A sharp signal at ca. 175.7 ppm arises from sugar COOH groups, and broad peaks ranging from 173 ~175 ppm arise from the C=O groups in the peptide/protein components; two peaks at ca. 130.5 and 116.6 ppm indicate aromatic and heteroaromatic ring carbons, such as the carbons from Phe (ca. 130 ppm), Try and His (117, 130 ppm), and Tyr (117, 130, 156 ppm). For these α-, β-, γ- and δ- carbons in amino acids, very broad peaks are given from 17 ~ 70 ppm, but mainly in three major regions: 18 ~ 20 ppm, 34 ~ 36 ppm and 52 ~ 55 ppm.

All three highly proteinaceous gums contain β-L-Araf as their major form of arabinose, and no α-L-Araf is apparent. The strong resonances at ca. 63.2 ppm (see Figs. 6.1, 6.2 and 6.3) suggest that most C_5 positions in β-L-Araf units are not involved in linkages. The methoxyl carbon in 4-O-Me-β-D-GlupA is sharp and high at around 60.0 ppm in confirmation of the high methoxyl contents. Free galactose and glucuronic acid are indicated in *Acacia tumida* gum (Fig. 6.2 and Table 6.2(b)) but

not free arabinose, and this may indicate that β-D-GlupA→β-D-Gal→β-D-Gal→ terminal groups exist in the structure.

6.4 By Smith-Degradation Studies

Smith-degradation procedures involve a periodate oxidation stage in which all sugar end groups and internal sugar units with diol or triol groups (such as 2,3,-diols or 3,4,-diols) within a polysaccharide structure are oxidised to give a polyaldehyde, which is then reduced by $NaBH_4$ to give the corresponding polyalcohol; controlled very mild acid hydrolysis of the polyalcohol, in which hydrolysis of the acyclic acetals from the cleaved sugar units occurs at room temperature without significant hydrolysis of glycosidic (even furanosidic) linkages, then results in the isolation of a degraded polysaccharide whose sugar residues resisted the oxidative cleavage by the periodate. Depending on the relative structural locations of such periodate-resistant sugar residues, the degradation may result in the formation of isolated units of low molecular weight in which the sugar residues are present as simple glycosides of fragments such as glycerol or tetritols, as well as in the formation of degraded polysaccharides from which further structural information concerning the inner structure of the original polysaccharide may be derived (Aspinall 1982).

6.4.1 Methods and Results

A. eriopoda, *A. difficilis* and *A. tumida* gums (5 g each) were each dissolved in water (100 ml) and 0.25M $NaIO_4$ solution was added (100 ml). The oxidation (in darkness, while stirring) was followed by measuring the release of formic acid with time. After 48 hours the reaction was stopped by adding ethylene glycol (3 ml); the solution was dialysed against running water for 2 days and then $NaBH_4$ was added; the mixture was kept for 30 hours then dialysed for a further 2 days. The polyalcohol was hydrolysed in N-sulphuric acid for 2 days after which the solution was neutralised (barium carbonate), filtered, deionised (Amberlite resin IR-120 (H^+)), reduced in volume to ca. 100 ml, and dialysed against distilled water (500 ml) for 24 hours, then dialysed continuously against running water for a further 2 days. Fraction SD1 was isolated as the freeze-dried product. The distilled-water dialysate was rotary evaporated and freeze-dried to obtain the "d-product" (dialysate). Only *A. tumida* gum was given a second Smith-degradation.

Table 6.3 The amino acid compositions (per 1000 residues) and analytical data for gums, the Smith-degradation degraded gums, and their degradation products

	From *A. eriopoda*			From *A. difficilis*			From *A. tumida*			
	er.	$SD1_{er}$	*erd*	*dif.*	$SD1_{dif}$	*difd*	*tu.*	$SD1_{tu}$	$SD1I_{tu}$	*tud*
Ala	27	24	47	33	37	68	31	33	26	61
Arg	23	16	28	25	19	30	25	25	17	27
Asp	92	100	78	81	115	83	81	93	86	86
Cys	35	9	17	44	14	16	25	9	0	11
Glu	30	28	59	40	52	90	83	96	43	118
Gly	43	32	130	53	51	214	59	61	55	90
His	13	13	49	25	7	23	19	16	18	20
Hyp	303	355	234	208	305	66	171	194	363	129
Ile	32	32	29	27	31	35	39	41	38	39
Leu	38	35	39	48	41	43	59	57	62	55
Lys	25	28	48	30	23	33	39	37	35	40
Met	5	2	2	7	0	3	4	3	3	4
Phe	25	20	23	32	25	28	31	31	37	28
Pro	50	44	52	55	36	61	58	53	14	55
Ser	100	115	107	104	106	106	93	97	78	104
Thr	50	53	42	50	45	40	63	68	60	60
Tyr	30	8	13	36	12	10	33	18	5	11
Val	77	86	58	76	82	52	66	70	59	64
NCF	6.79	6.78	5.96	6.85	6.32	5.85	6.75	6.75	6.91	6.75
N%	6.8	7.2	4.5	8.5	8.7	6.4	6.7	6.9	7.2	3.44
Rec%	-	37	16	-	66	10	-	62	[a]43	10
Neutral sugar ratios after hydrolysis%										
Gal	19	45	26	46	55	30	48	62	68	23
Ara	81	55	74	54	45	70	52	38	32	77
Rha	<1	0	0	<1	0	0	0	0	0	0

[a]: 43% of SD1
"*er*" for *A. eriopoda* "*dif*" for *A. difficilis* "*tu*" for *A. tumida*

6.4.2 Discussion

All those three highly proteinaceous gums have slightly more protein contents in their SD1 products than in the original gums. Data in Table 6.3 show that the galactose content of the degraded gums is higher than in the original gums, and in each case the SD products contain enhanced proportions of Hyp. Fig. 6.4 shows the ^{13}C NMR spectrum of the Smith-degraded *A. eriopoda* gum. As shown in Table 6.3, the arabinose content had been greatly reduced, as is confirmed by comparison with Fig 6.3 (original gum). Many of the original internal β-L-Arap residues (ca. 97 ppm in Fig. 6.3) have disappeared after the Smith-degradation (Fig. 6.4), but a high protein content can still be observed, as was confirmed later by the analytical data for the

nitrogen content. The structure of the *A.eriopoda* SD1 product largely consists of terminal β-L-Araf (101.5, 81.8, 76.3, 73.2, 63.2 ppm), internal (1→3) linked β-D-Gal (103.6, (70.2), 81.8, (68.6), 73.2, (69.3) ppm), and internal (1→2) linked β-L-Araf (98.6 and 83.4 ppm for C_1 and C_2), together with the remaining unattacked (1→3) linked β-L-Arap (98.6 and 78.6 ppm for C_1 and C_3). The large peaks between 61.0 and 62.6 ppm may be caused by erythritol residues or threitol which may remain linked with the main structure (Goldstein et al. 1965).

The *A. difficilis* degraded gum SD1 shows similar results to *A. eriopoda* degraded gum (Fig. 6.7), but with an even higher proportion of galactose in the product SD1 after the Smith-degradation. Although terminal β-L-Araf is observed (101.4, 81.8, 76.0, 72.6, 63.1 ppm), there is clearly more galactose (mainly 1,3,6 linked β-Gal) than arabinose in the structure in agreement with the sugar analysis data. From the spectrum, it is seen that there is a large amount of GlupA with major (1→4) links in SD1 (C_1 at 102.0, C_2 at (73.4), C_3 at (74.1), C_4 at 79.0, C_5 at 76.0, C_6 at 173 ppm) but the C_2 and C_3 signals are much lower than for the other carbons.

Relatively high nitrogen contents were found in the dialysate fractions "*er*", "*dif*" and "*tu*" (N%= 4.5, 6.4, 3.4 respectively). The data also show that the Gly, Glu and Ala contents are increased and that Hyp, Tyr and Val are decreased in the dialysates (d-products); the other amino acids remain without significant changes in their relative proportions. This suggests that Gly, Glu and Ala may be more closely associated with the periodate-vulnerable sugar units than the other amino acids; in other words, located at peripheral positions of the structures. The amino acid composition of the Smith-degradation products also reveals that the Hyp content is increased after degradation carried on; this is most strongly evident in the SD2 product from *A. tumida* gum. This therefore supports previous evidence (Anderson and McDougal 1987a) that Hyp is of major importance in the inner branched galactan core of *Acacia* gums. The presence of high nitrogen contents in the degraded gums and dialysates indicates that the amino acids (in peptides more probably than proteins) are distributed throughout the macromoleculer gum structure, from the periphery to the core.

Intact galactose and arabinose have also been found (Table 6.3) in the dialysate products after the Smith-degradation. This suggests that certain internal sugar units may be linked with the side-chain (or terminal groups) which was unattacked (or unable to be attacked) by IO_4^-, indicating that these side-chains do not have diol groups and that their carbons are involved in linkages; and that these

A. difficilis gum (SD1)

ppm	Intensity
173.22	5.2
171.60	4.6
128.78	4.1
103.89	5.1
102.00	7.7
101.44	3.4
98.60	2.8
82.12	7.0
81.78	5.5
79.02	16.5
76.04	7.3
72.65	4.8
70.02	11.7
68.56	6.4
67.10	4.5
63.12	8.2
62.64	24.0
61.89	8.2
61.45	25.7
61.12	31.0
60.56	2.7
59.44	7.2
59.13	8.2
57.45	10.2
55.13	6.1
53.89	7.0
53.03	5.3
50.67	5.7
37.13	7.9
35.89	8.9
30.14	5.5
24.45	4.4
22.20	4.6

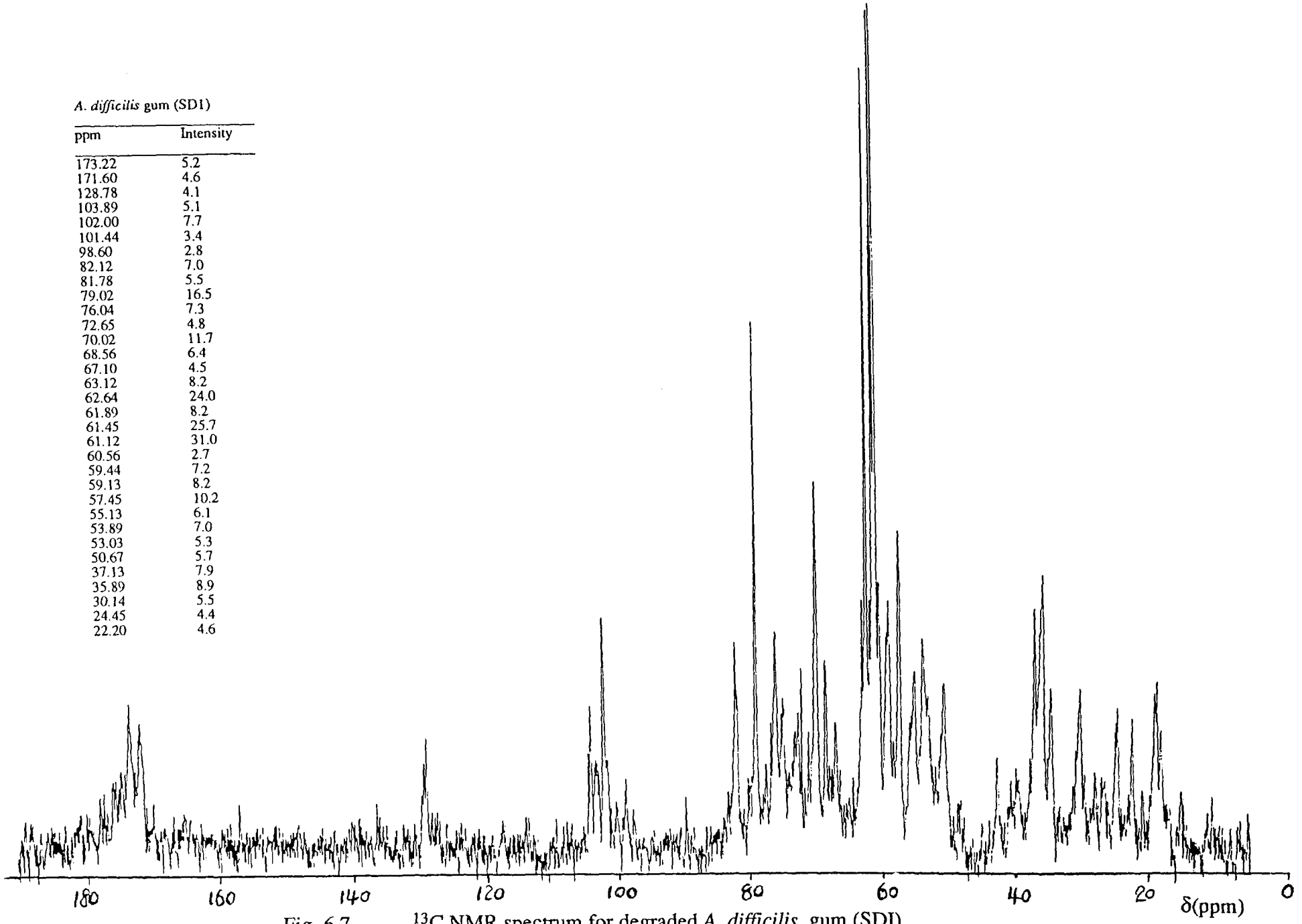

Fig. 6.7 ^{13}C NMR spectrum for degraded *A. difficilis* gum (SDI)

periodate-vulnerable sugars may be linked with very large molecular weight blocks (Churms and Stephen 1984).

6.5 By Gel Permeation Chromatography (GPC)

In gel permeation techniques, polysaccharide or protein molecules are separated according to size on a column packed with a selected porous medium. Molecules larger than the largest pores in the medium, those above its exclusion limit, cannot enter the medium and therefore are eluted first; smaller molecules can enter the pores of the packing medium to varying extents depending on their size and shape and are therefore slowed down during their passage through the column. Molecules are eluted in a predictable way in order of decreasing molecular size. This technique, also known as Gel Filtration and Size Exclusion Chromatography, is a non-destructive method for separating macromolecules such as polysaccharides and proteins, and it can be used to estimate molecular weights if a column calibration can be achieved with a series of valid known molecular weight standards applied under the same chromatographic conditions.

6.5.1 Methods and Results

GPC was used to separate *A. eriopoda* and *A. difficilis* gums into various molecular mass fractions. A glass column of dimension 2.4 x 90 cm was packed with Sephacryl S-500 gel (Pharmacia). 20 ml of 5% *A. eriopoda* gum solution in distilled water was filtered through a Whatman No.1 filter paper, added to the column, and eluted under gravity using distilled water as eluent at room temperature. Fractions were collected (ca. 4 ml/tube) and screened by the phenol-sulphuric acid method in order to obtain an elution profile (Fig. 6.8). As a result, four freeze-dried fractions were obtained, viz. $er._{L}$, $er._{M}$, $er._{m}$ and $er._{S}$, their molecular mass progressively decreasing.

The same procedure was applied to *A. difficilis* gum; 3 fractions were obtained, $dif._{L}$, $dif._{M}$ and $dif._{S}$.

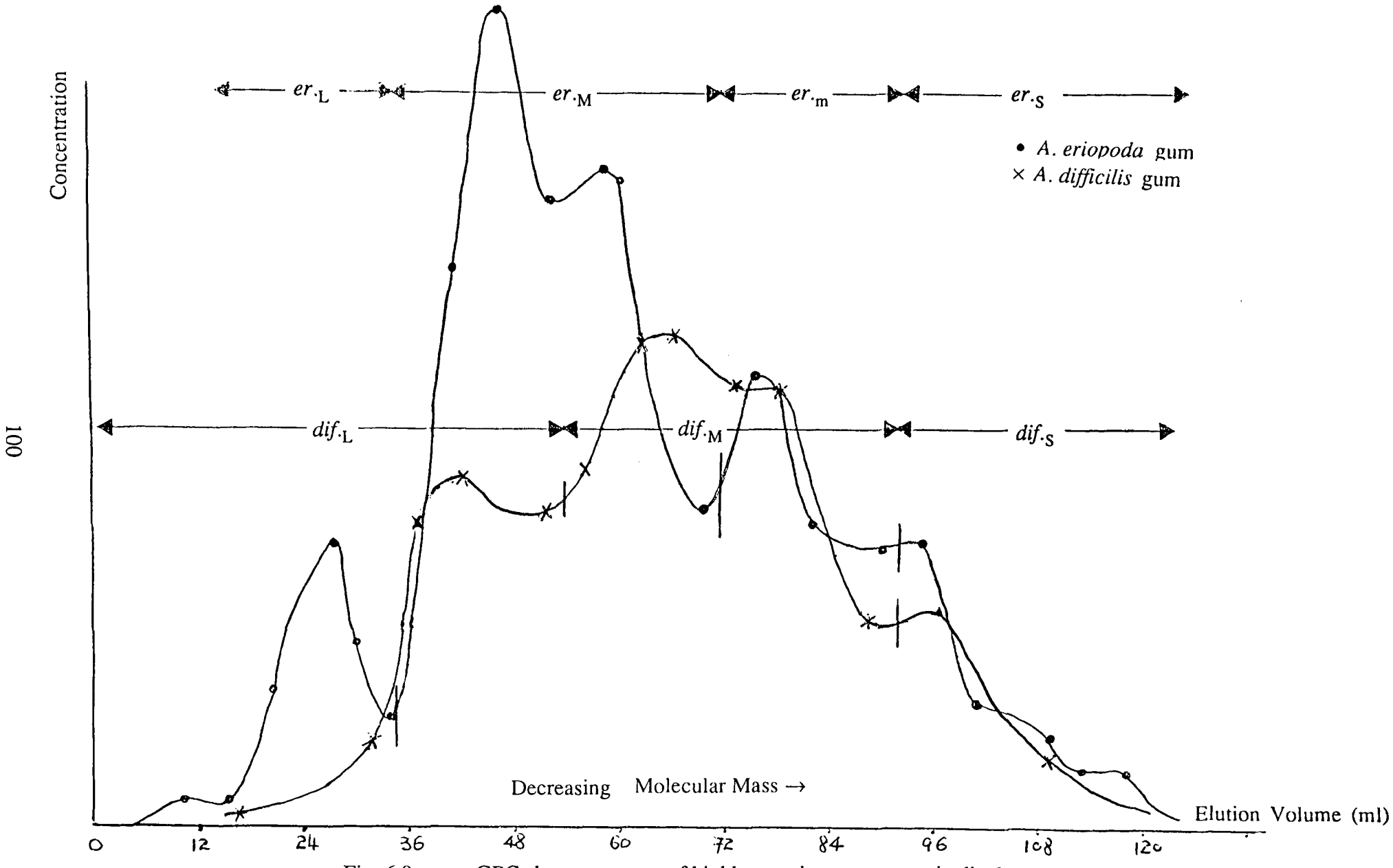

Fig. 6.8 GPC chromatograms of highly proteinaceous gums in distd. water as detected by phenol-sulphuric acid method at 430 nm

Table 6.4 The amino acid compositions (per 1000 residues) and analytical data for the GPC fractions

	From *A. eriopoda*					From *A. difficilis*			
	*er.**	$er._L$	$er._M$	$er._m$	$er._S$	*dif.**	$dif._L$	$dif._M$	$dif._S$
Ala	27	19	22	25	30	33	28	35	41
Arg	23	19	20	24	25	25	23	23	28
Asp	92	79	85	92	95	81	79	83	91
Cys	35	22	39	38	33	44	37	42	65
Glu	30	22	28	33	29	40	33	39	42
Gly	43	33	41	47	57	53	46	54	62
His	13	12	12	13	13	25	23	27	21
Hyp	303	392	334	280	208	244	289	266	176
Ile	32	22	26	31	36	27	25	26	30
Leu	38	29	34	42	49	48	41	48	54
Lys	25	20	23	29	35	30	31	29	33
Met	5	4	5	5	5	7	7	7	8
Phe	25	19	24	30	33	32	26	32	35
Pro	50	46	50	50	53	55	53	57	56
Ser	100	108	102	102	95	104	103	105	94
Thr	50	48	51	51	52	50	50	51	55
Tyr	30	27	29	32	29	36	31	35	40
Val	77	78	75	79	77	76	75	81	69
NCF	6.69	7.03	6.98	6.88	6.79	6.85	6.76	6.68	6.63
N%	6.8	5.1	6.4	6.5	6.8	8.5	8.8	7.7	7.6
Rec%	-	10	30	20	22	-	27	46	10
Neutral sugar composition after hydrolysis%									
Gal	19	17	19	25	36	46	43	55	22
Ara	81	83	81	75	64	54	57	45	78
Rha	<1	0	0	0	0	<1	0	0	0
[η]ml/g	19	n.d.	n.d.	n.d.	n.d.	30	25	21	18
$[\alpha]_D$	+5°	n.d.	n.d.	n.d.	n.d.	-10°	n.d.	-40°	-11°

* Data from Tables 6.3 and 6.1.

6.5.2 Discussion

Table 6.4 shows that each fraction, both from *A. eriopoda* gum and *A. difficilis* gum, still contains quite a high nitrogen content in comparison with the original gums in spite of their different molecular size. The molar ratios of neutral sugars after acid hydrolysis of the fractions in *A. difficilis* gum show that the smaller molecular weight fractions contain a much higher proportion of arabinose than galactose, whilst in the fractions of *A. eriopoda* gum, the smaller fraction has the lower proportion of arabinose. Only Hyp and Ser decrease with increasing molecular weight; the proportions of most other amino acids remain unchanged.

Two ^{13}C NMR spectra were obtained for *A. difficilis* gum GPC fractions (Figs. 6.9-6.10). High protein contents still can be observed from both spectra (around 116, 130 ppm). $er._L$ contains more terminal β-L-Araf, which is the only form of arabinose in this fraction, than $er._M$ (82.2-81.9 ppm for C_4 and 100.0-100.2 ppm for C_1). Comparison of the C_1 intensity of β-L-Araf with that of β-D-Gal, indicates that the ratio of Gal:Ara in $er._M$ is much higher than in $er._L$. In addition, free arabinose signals appear in the $er._M$ spectrum (Fig. 6.10) (96.7, 92.5 ppm for α and β C_1; 66.3 and 62.4 ppm for α and β C_5), but there are no free galactose signals. This indicates that the arabinose is in peripheral positions.

6.6 By Enzymatic Degradation

6.6.1 Material and Methods

1 ml of protease from Rhizopus species (Sigma No.p-5027) was added to 100 ml 5% *A. difficilis* gum solution held in a cellophane membrane bag (molecular cut-off 3,000 daltons) and dialysed against 500 ml distilled water in a beaker at 37°C for 24 hours at natural pH. The dialysate was then collected and reduced in volume to ca. 1 ml for free amino acid analysis i.e. without 6N HCl hydrolysis. This experiment was repeated with two other enzymes, protease (Sigma No.p-6911) (pronase E from Streptomyces griseus) and Viscozyme 120L(carbohydrase complex) (NOVO).

6.6.2 Results and Discussion

Table 6.5 shows the free amino acid compositions obtained from *A. difficilis* gum after being treated by the three enzymes. Data for the whole gum's amino acid composition, after hydrolysis, are included for comparison. Although the free amino acid compositions released by the enzymes differ greatly from that of the whole gum, there is a great similarity between the free amino acids released by the proteases except for Glu which is not found in $dif._{p6911}$ (the different proteases did not all function in exactly the same way). Treatment with proteases leads to the release of Ala, Ile, Leu, Lys, Met, Phe, Try and Val but not of Asp, Cys and Hyp. The differences between the free amino acid compositions released by the proteases and by the carbohydrase are that $dif._v$ contains higher Arg, Gly and His but lower Leu, Met and Ser, and no Pro; in contrast, $dif._{p5027}$ and $dif._{p6911}$ give, in addition, some Pro. However all of the enzymes used released relatively large amounts of Ala, Ile, Leu, Lys, Tyr and Val and much lower amounts of Asp, Cys, Glu, Hyp and Pro. This indicates that some amino acids in this gum are more susceptible than others to

A. difficilis gum GPC fraction $dif._L$

ppm	Intensity
173.31	2.7
171.64	2.7
130.78	4.4
116.56	5.0
104.20	8.9
103.89	9.2
103.34	10.5
103.10	12.3
100.21	15.8
82.23	31.0
81.52	22.7
76.54	29.6
75.00	23.2
74.67	20.1
73.65	28.3
72.89	24.9
71.03	8.2
70.22	13.5
69.89	14.7
68.67	14.3
63.44	23.9
61.23	9.3
60.42	6.5
60.33	11.6
59.24	7.4
57.56	10.8
53.02	6.7
34.78	8.3

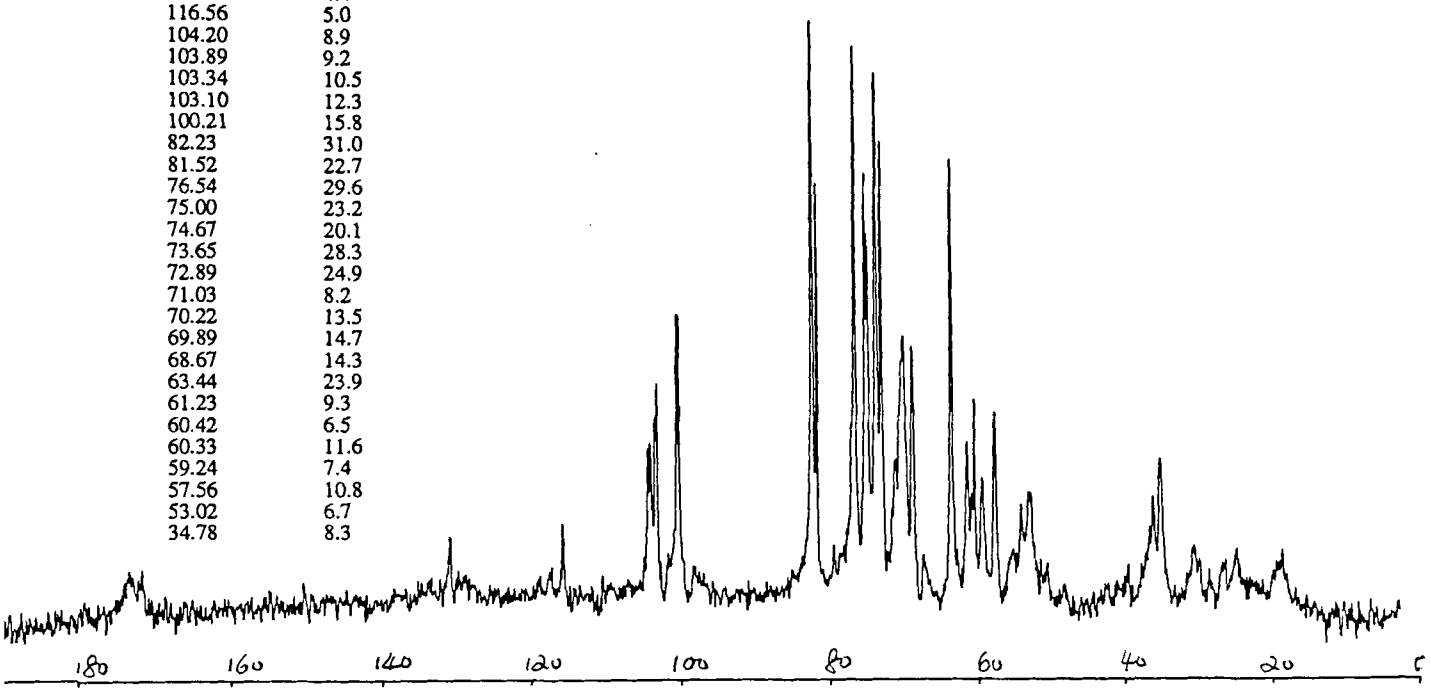

Fig. 6.9 ^{13}C NMR spectru

A. difficilis gum GPC fraction $dif._M$

ppm	Intensity	ppm	Intensity
173.22	3.3	74.78	23.7
172.56	4.1	74.43	15.2
172.40	3.3	73.25	28.4
130.62	3.3	72.67	31.8
115.31	4.1	72.44	17.9
104.10	6.7	69.89	22.6
103.89	6.0	68.41	25.3
103.67	7.3	66.32	12.2
103.20	10.4	63.21	11.0
102.93	12.4	62.43	6.6
101.43	3.2	61.14	12.2
100.02	7.6	60.20	16.8
96.72	7.2	59.04	6.9
92.53	3.7	57.55	8.2
81.89	15.0	57.34	8.7
81.33	24.9	35.67	5.9
76.25	15.3	18.21	4.7

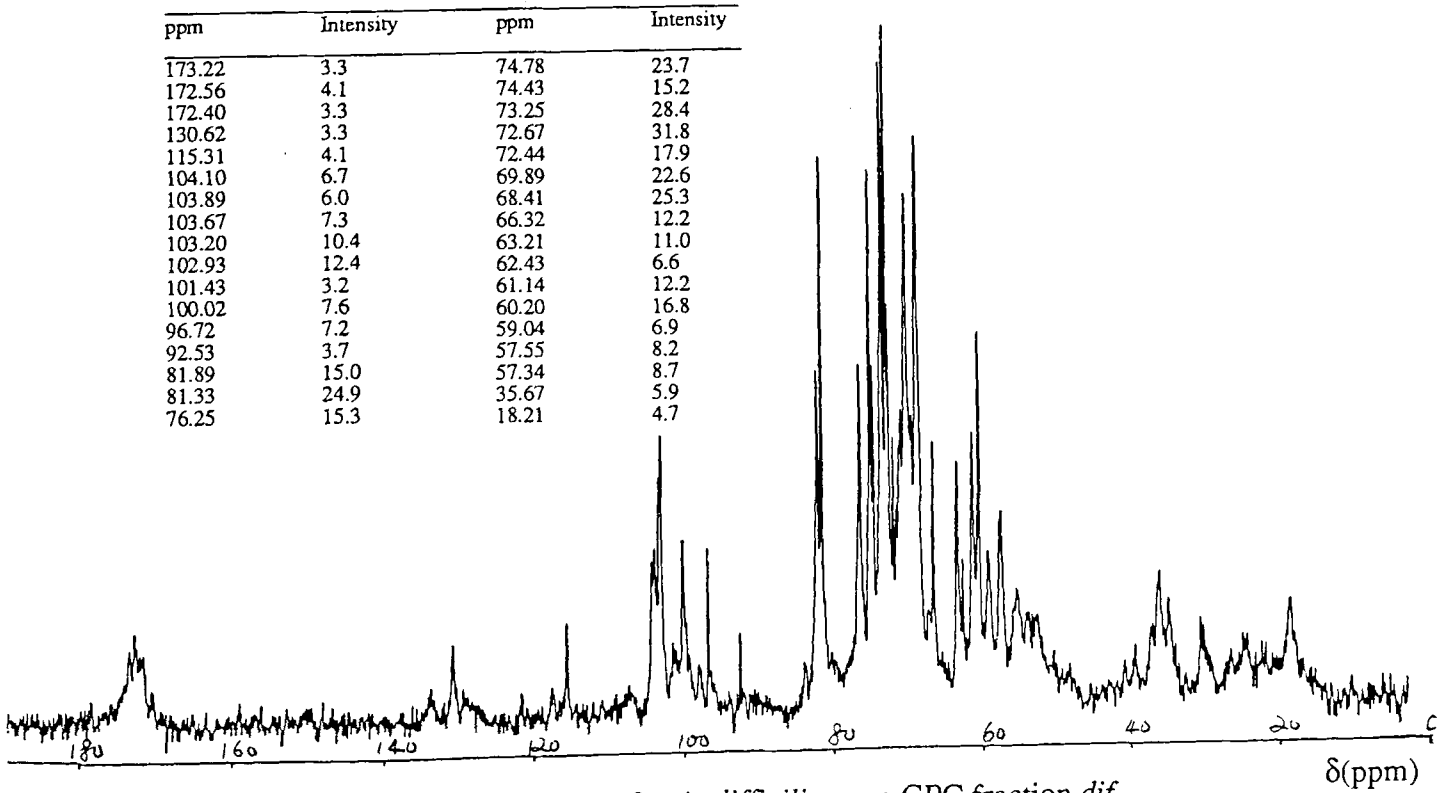

Fig. 6.10 ^{13}C NMR spectrum for *A. difficilis* gum GPC fraction $dif._M$

enzyme attack, and appears to confirm that Hyp, Cys and Asp, in particular, are associated with the core of the gum rather than its periphery, a result previously demonstrated for *A. senegal, A. seyal* and *A. polyacantha* gums, and also for the highly proteinaceous gums, by the Smith-degradation method.

Table 6.5 Amino acid composition (residues per 1000) for free amino acids dialysed from *A. difficilis* gum treated by enzymes.

	Enzyme treated			Whole gum
	dif.$_{p5027}$	*dif.*$_{p6911}$	*dif.*$_{v}$	*dif.*
Ala	65	70	64	33
Arg	23	23	45	25
Asp	0	1	2	81
Cys	0	0	0	44
Glu	22	0	5	40
Gly	43	54	77	53
His	15	11	58	25
Hyp	0	0	0	234
Ile	75	76	78	27
Leu	117	114	85	48
Lys	219	218	205	30
Met	13	13	2	7
Phe	65	58	54	32
Pro	24	24	0	55
Ser	115	118	84	104
Thr	42	45	40	50
Tyr	219	218	205	36
Val	109	120	132	76
NCF	7.32	7.21	6.34	6.85

Chapter 7
Studies of Leucaena Gums

7.1 Introduction

Leucaena, from the Greek *leukos*, means "white" in reference to the colour of the flower. Members of this genus are widespread in tropical and subtropical North and South America, Africa and Polynesia. Species are both wild and cultivated and they are well adjusted to a variety of soils, waste lowlands, wet and dry areas (Allen and Allen 1981). From 1977, some publications (US National Academy of Science 1977; 1979; 1980) drew attention to the diverse uses and attractive ecological properties of a tropical leguminous genus, *Leucaena*, which provides a valuable source of proteinaceous fodder for animals, edible beans for humans with crude protein content as high as 27.5% (Azeemoddin et al. 1988), rapidly growing timber, wood-pulp and firewood. *Leucaena* trees also fix nitrogen symbiotically. One species, namely *Leucaena leucocephala (Lam.) De Wit* has already been described as "a promising versatile leguminous tree for the tropics" (Blom 1981); it can derive about 65% of its total nitrogen requirement from N_2 fixation compared to about 20% by *Acacia albida* (Sanginga et al. 1990). At least 100 different *Leucaena* species, based on three main types ascribed to Hawaii, Salvador and Peru, are known; they show great genetic diversity. Plantations of *Leucaena spp.* have now been established in many tropical and subtropical locations throughout the world, principally to increase local firewood production.

The potential agroforestry developments in *Leucaena* production and the observation that *Leucaena spp.* may yield gum exudates under certain conditions led to a study which reported that gum from a *Leucaena leucocephala* cultivar growing in Southern India showed physico-chemical properties and analytical parameters that were very similar to those of gum arabic (Anderson et al. 1983c). More analytical data were published for different *Leucaena* species from Hawaii, Mexico and South India later (Anderson and Brown Douglas 1988; 1989). This Chapter shows the analytical data for this genus from *Leucaena spp.* growing in Honduras and Guatemala. In addition, their ^{13}C NMR spectra were obtained in order to unravel their structural differences from *Acacia senegal* gum and to elucidate the possible structure of *Leucaena* gums.

7.2 Origin of Gum Samples

The *Leucaena* gum samples were kindly provided by Colin Hughes, Research Officer, Department of Plant Science, University of Oxford.

Gum sample identification code:

"Lsp" for an unnamed *Leucaena sp.* gum from Aguan Valley, Yoro, Honduras; 15°28'N, 86°04'N, Alt. 180m was collected in February 1991.

"Lsh" for gum from *Leucaena shannonii subsp. shannonii*, from Asuncion Mita, Jutiapa, Guatemala; 14°25'N, 89°41'W, Alt. 700m was collected in March 1991.

"Lc" for gum from *Leucaena collinsii subsp. zacapana* tree from Ipala, Chiguimula, Guatemala; 14°34'N, 89°32'W, Alt. 900m was collected in January 1991.

"Le" for gum from *Leucaena esculenta subsp. esculenta* from cultivated material in Comayagua, Honduras, from an original Mexican seed source; 18°18'N, 99°45'W, Alt. 1550m was collected in January 1991.

"Ld" for gum from *Leucaena diversifolia subsp. stenocarpa* from Copan, Honduras; 14°50'N, 89°10'W, Alt. 550m. was collected in March 1991.

"Ll_1 and "Ll_2 " for gums from *Leucaena leucocephala (Lam.) De Wit*, from Auroville, Pondicherry, South India and from Waimanolo, Hawaii respectively; and "GA_{su}" for Sudanese gum arabic are included for comparison purposes.

7.3 Results and Discussion

The exudation of *Leucaena* gum has been reported to occur in Indian, Hawaiian and now in Central American species. There are therefore indications that there may be future possibilities, in at least some location, for economic advantages for natives to be obtained through the collection of *Leucaena* gum for industrial/technological uses (not food uses); e.g *Leucaena glauca* gum solutions were shown to have pseudoplastic flow and that transition from Newtonian to pseudoplastic behaviour occurred in the low shear rate range at concentrations of interest to industry (Raval et al. 1988).

Table 7.1 Analytical data for *Leucaena* gum samples from Central America

Items	Gum sample							
	Ll_1*	Ll_2*	Lsp	Lsh	Lc	Le	Ld	GA_{su}
H_2O%	17.7	24	13.1	10.5	14.6	12.3	11.5	13
Ash%	4.4	6.3	4.1	4.4	5.6	4.0	3.9	2.6
N%	0.37	0.52	0.44	0.40	0.69	0.47	0.38	0.34
NCF	6.67	6.74	6.72	6.64	6.71	6.64	6.81	6.62
Protein%	2.5	3.5	3.0	2.7	4.6	3.1	2.6	2.3
Methoxyl%	0.92	0.71	0.15	0.32	0.5	0.1	0.7	0.25
$[\alpha]_D$	-28°	-35°	-17°	-19°	-12°	-42°	-40°	-30°
[η]ml/g	24	20	19	15	15	15	13	16
E.Wt[a]	770	690	1145	1020	800	1100	1375	1050
U.A.A.	23	26	15	17	22	16	13	17
Solubility in water%	100	100	98	100	100	98	70	100
Sugar composition after hydrolysis%								
4-O-MGUA[b]	5.5	5	1	2	3	1	4	1.5
GalA	0	0	0	0	0	0	0	0
GUA	17.5	21	14	15	19	15	9	15.5
Gal	36	48	47	43	24	40	36	44
Ara	22	17	27	28	45	32	43	25
Rha	19	9	11	12	9	12	8	14
Amino acid composition (per 1000 residues) for *Leucaena* gum samples								
Ala	59	46	51	54	52	53	45	27
Arg	11	18	20	19	21	16	16	13
Asp	64	50	59	56	50	55	56	68
Cys	1	0	0	0	26	0	0	2
Glu	30	28	32	43	40	29	28	42
Gly	29	33	28	30	38	27	28	50
His	37	32	30	34	28	36	26	44
Hyp	306	386	324	319	338	291	326	304
Ile	27	21	21	50	26	16	33	12
Leu	38	42	47	31	34	55	42	66
Lys	34	27	23	35	26	36	30	25
Met	0	3	2	2	2	2	2	2
Phe	10	10	16	8	8	11	15	33
Pro	74	60	94	75	68	93	102	63
Ser	164	145	156	142	139	159	147	129
Thr	43	37	31	36	32	46	35	68
Tyr	31	32	34	36	41	45	40	14
Val	40	30	32	30	31	30	29	35
NCF	6.67	6.74	6.72	6.64	6.71	6.64	6.81	6.62

* Indian and Hawaiian *L. leucocephala* gums (Anderson and Brown Douglas 1988).
a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).
GA_{su} Sudanese gum arabic mean values(see Table 4.6)

The analytical data in Table 7.1 show that Central American *Leucaena* gums have amino acid composition, nitrogen and ash contents similar to those for *Leucaena* gums from India and Hawaii (Anderson and Brown Douglas 1988). In comparison with gum arabic, nitrogen contents for *Leucaena* gum tend to be slightly higher with Hyp, Ser and Pro as the most abundant amino acids in its composition. In addition, Ala, Ile, Pro and Tyr are always higher, with Gly, His, Phe and Thr always lower in *Leucaena* gum than in gum arabic; the ratio of Ile:Leu in *Leucaena* gum (7:10) is much higher than in gum arabic (2:10).

The specific rotation of *Leucaena* gums ranges from -17° to -40°. The methoxyl content in *Leucaena* gums is generally higher than that of gum arabic. The intrinsic viscosities for Central American *Leucaena* samples are slightly lower than those for *Leucaena* gum from India and Hawaii and close to that of gum arabic. The sugar compositions of the *Leucaena* gums have higher proportions of galactose than arabinose, except for *Leucaena collinsii* and *Leucaena diversifolia* gums.

Fig. 7.1 shows a typical *Leucaena leucocephala* gum ^{13}C NMR spectrum. When compared with the spectrum for *Acacia senegal* gum it is seen that *Leucaena leucocephala* gum contains both α and β L-Araf, while only the former exists in gum arabic. The spectra for *Leucaena leucocephala* and *A. senegal* gums are distinctly different.

Table 7.2 The composition of sugar units indicated by some assignments in the ^{13}C NMR spectrum for *Leucaena leucocephala* gum (Fig. 7.1)

Chemical shift (ppm)	Intensity	C_1 of
109.3	6.3	α-L-Araf(1→
103.7	6.2	→β-D-Gal(1→ and β-D-Gal(1→
102.6	10.9	→β-D-GlupA(1→ and β-D-GlupA(1→
101.3	12.6	→3)β-L-Araf(1→ and
β-L-Araf(1→		(with 62.9 ppm from C_5)
100.6	12.6	α-L-Rham(1→
62.8	11.4	β-L-Araf C_5
61.2	9.2	β-Gal C_6 and α-Araf C_5
59.9	2.3	$-OCH_3$ in 4-O-Me-β-D-GlupA
16.4	15.2	α-L-Rham C_6

Table 7.2 lists the anomeric peaks (from 100.6 to 109.3 ppm) for *L. leucocephala* gum and supporting C_5 and C_6 assignments of the constituent sugars. 101.3 ppm is

L. leucocephala gum

ppm	Intensity
109.35	6.3
103.71	6.2
102.55	10.9
101.33	12.6
100.66	12.6
83.59	6.8
82.65	8.2
81.90	16.5
80.03	7.4
78.98	10.6
76.20	16.6
74.96	5.5
74.08	24.4
73.23	20.3
71.86	15.0
71.56	6.9
70.18	19.9
69.94	25.2
68.86	22.4
62.81	11.4
61.19	9.2
59.97	2.3
16.44	15.2

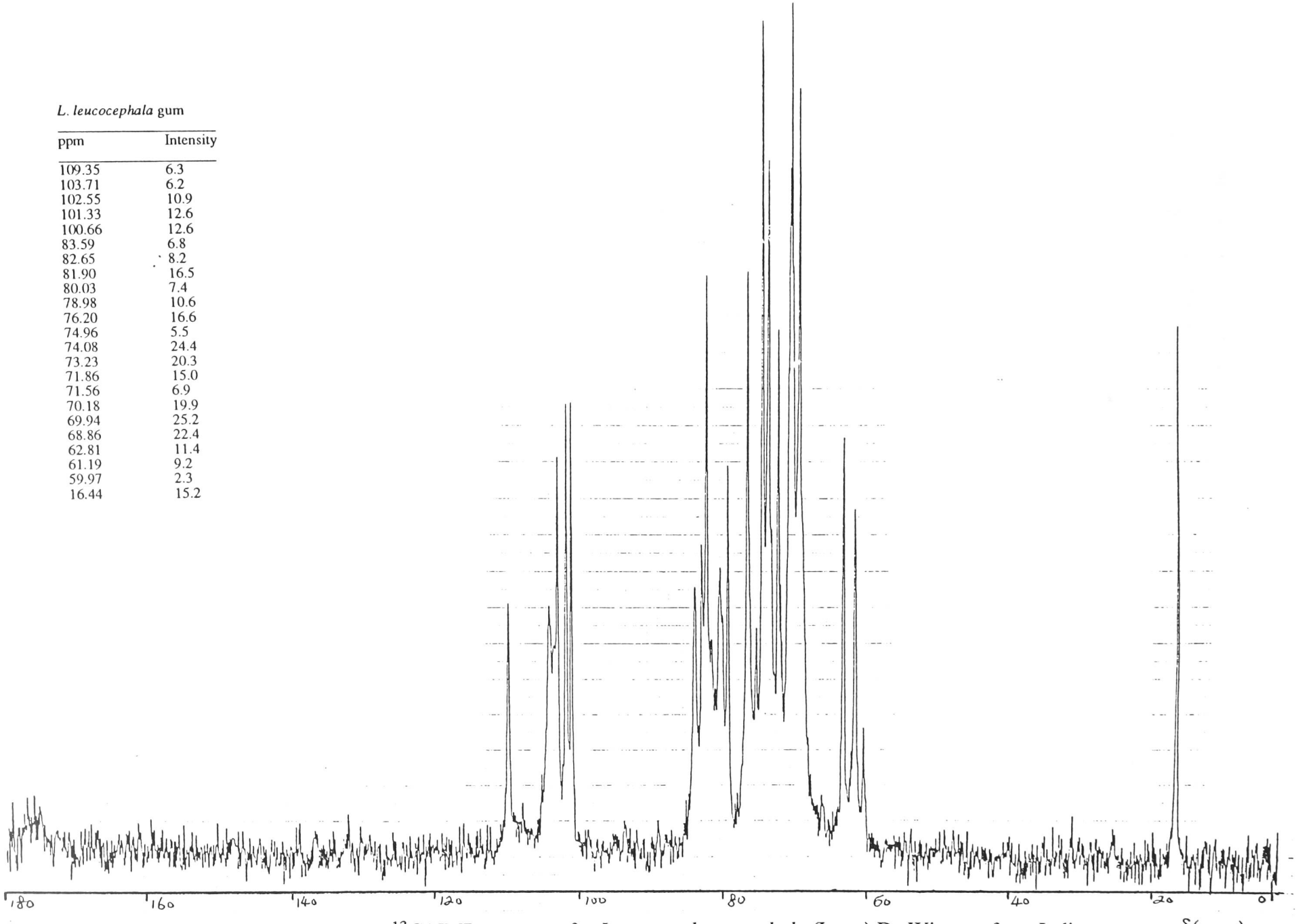

Fig. 7.1 ^{13}C NMR spectrum for *Leucaena leucocephala* (Lam.) De Wit gum from India

for C_1 of β-L-Araf, which is possibly involved in 1→3 linkages, and 62.8 ppm represents its C_5 resonances. The 59.9 ppm signal arises from the 4-OCH_3 groups in GlupA, and the higher proportions of rhamnose in *Leucaena leucocephala* gum are shown at 16.4 ppm.

All Central American *Leucaena* gums also have β-L-Araf present in their structural compositions as shown by their ^{13}C NMR spectra (Figs. 7.2 - 7.6(a)); some gums contain more β-L-Araf than others. In addition, all of the spectra also show that various amounts of free galactose and arabinose exist in those gums; the four peaks around 96.7, 96.5, 92.5 and 92.3 ppm represent C_1 of α-L-Arap, β-D-Galp, β-L-Arap and α-D-Galp respectively (Table 7.3).

Table 7.3 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectra for *Leucaena* gums from Central America

Chemical shift (ppm)	C_1 of
109.3	α-L-Araf(1→
103.7-102.9	→β-D-Gal(1→ and β-D-Gal(1→
102.6	→β-D-GlupA(1→ and β-D-GlupA(1→
101.3 -101.5	→3)β-L-Araf(1→ and β-L-Araf(1→ (with 62.9 ppm from C_5)
100.5	α-L-Rham(1→
96.7	free α-L-Arap
96.4	free β-D-Gal
92.5	free β-L-Arap
92.3	free α-D-Gal

By further careful examination, a very small amount of free glucuronic acid (at 95.72 and 92.07 ppm for β-D-GlupA) can be detected, but no trace of free rhamnose is evident. This may indicate that a proportion, at least, of galactose and arabinose are present at peripheral positions and are easy to degrade naturally. Fig. 7.6(b) shows the ^{13}C NMR spectrum of *Leucaena diversifolia* gum recovered after an aqueous solution was dialysed against distilled water: the free galactose and arabinose peaks have disappeared drastically in comparison with Fig. 7.6(a); in the solution of the dialysate, without acidic hydrolysis treatment, the presence of free galactose and arabinose was shown by paper chromatography. Since no free rhamnose was found in the gum, the rhamnose probably occurs in internal linkages. (1→3) α-L-Rham and side-chain arabinose, which were reported in *Leucaena leucocephala* gum (Soni et al. 1991), may be common in most *Leucaena* gums and side-chain galactose and Araf-Gal branches are also obviously present in *Leucaena* gums.

L. sp. gum

ppm	Intensity	ppm	Intensity
175.04	1.0	74.07	11.9
109.27	1.4	73.59	13.3
103.99	2.1	73.19	12.5
103.29	4.7	72.67	10.4
103.04	4.7	72.48	15.1
102.45	5.3	72.35	14.1
102.24	6.7	71.78	25.1
101.30	1.5	70.64	10.9
100.60	11.4	70.17	15.0
99.23	0.8	69.95	15.1
96.63	8.0	69.69	7.3
96.32	10.3	69.20	19.6
92.45	4.2	68.82	20.1
92.19	4.6	68.63	23.0
83.77	1.2	68.51	21.5
82.59	2.7	68.40	20.3
81.85	3.9	67.29	1.1
81.22	1.9	66.26	11.7
79.73	1.7	62.81	1.6
78.88	10.3	62.35	6.8
75.69	8.0	60.91	10.6
75.00	9.2	16.43	9.8

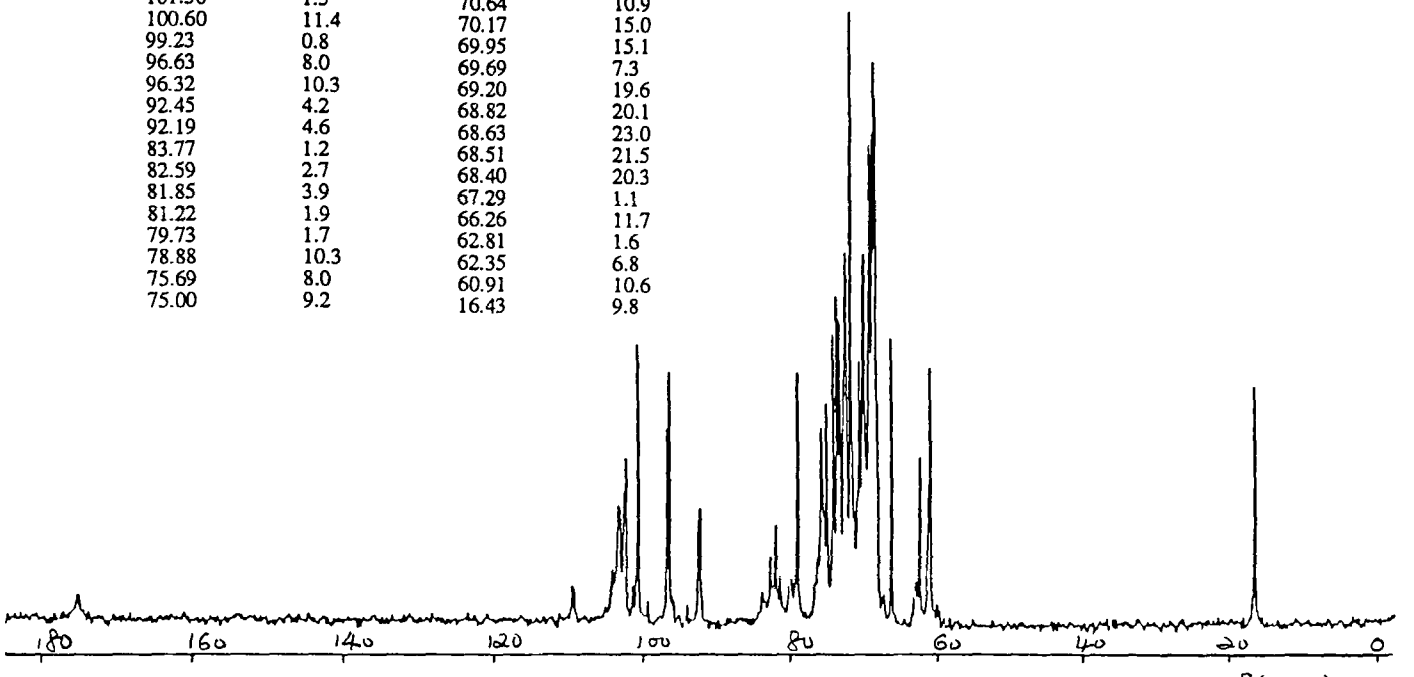

Fig. 7.2 ^{13}C NMR spectrum for *Leucaena spp.* gum from Honduras

L. shannonii gum

ppm	Intensity	ppm	Intensity
109.32	6.8	73.27	21.1
103.38	9.1	72.90	10.3
103.19	9.1	72.57	15.5
102.58	12.6	71.89	20.3
101.38	10.1	71.64	7.6
100.66	14.4	71.41	5.6
96.72	2.0	70.71	16.2
96.39	2.2	70.50	10.5
83.56	6.7	70.26	22.1
82.65	9.2	70.03	23.2
81.94	16.6	69.76	18.3
81.31	3.7	69.29	19.8
80.12	7.0	68.90	25.0
79.79	7.4	68.60	24.3
78.99	13.9	66.33	4.0
76.25	12.1	62.87	11.0
75.90	9.2	62.44	2.7
75.01	6.2	61.28	13.0
74.17	22.3	60.00	1.7
73.64	19.0	16.50	11.4

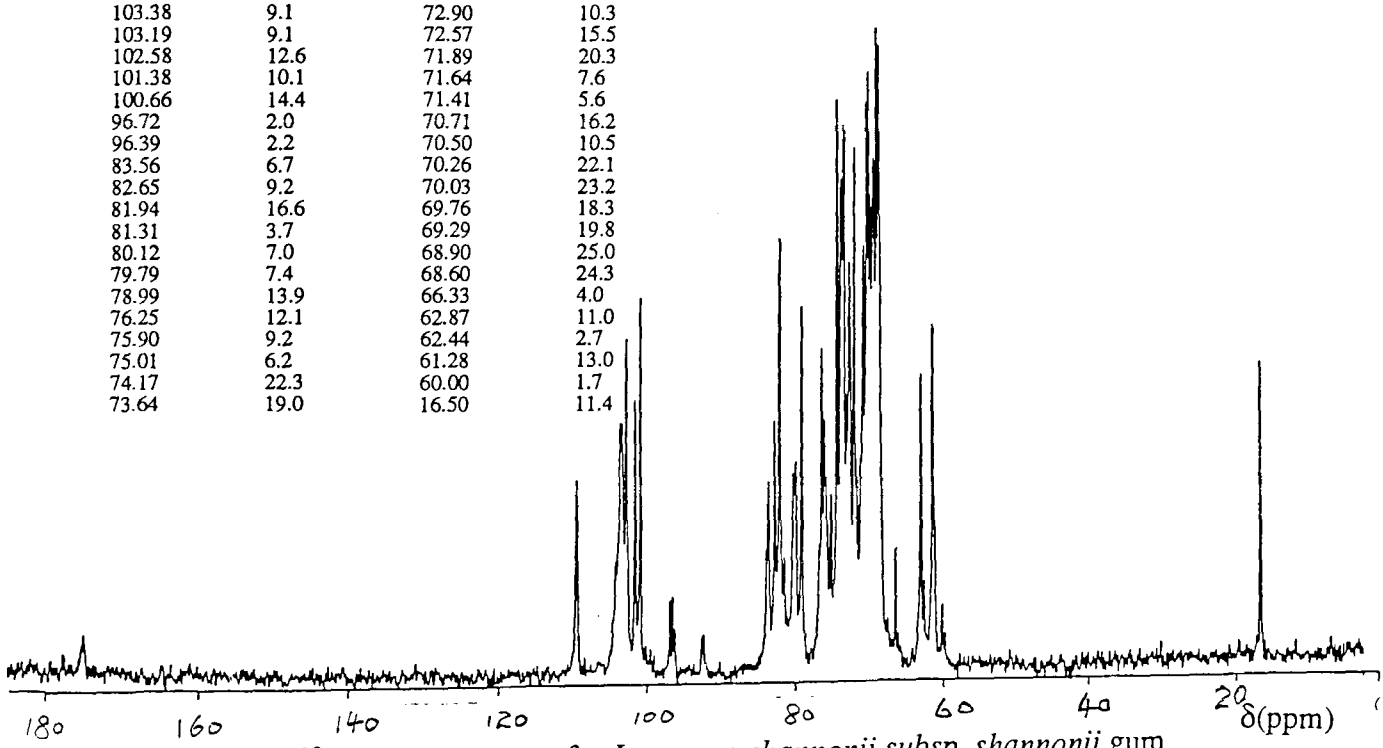

Fig. 7.3 ^{13}C NMR spectrum for *Leucaena shannonii subsp. shannonii* gum

L. collinsii gum

ppm	Intensity	ppm	Intensity
175.05	2.5	73.67	18.5
109.32	6.5	73.34	20.1
103.70	6.4	72.97	16.2
103.12	9.8	72.64	16.3
102.61	14.0	71.97	14.6
101.62	10.0	71.65	14.3
100.52	8.3	71.45	12.8
96.75	3.8	70.71	14.5
96.42	6.7	70.32	15.3
92.51	1.8	69.77	13.8
92.34	4.5	69.35	15.1
83.46	6.4	68.92	17.8
82.60	8.9	68.64	21.0
81.97	25.1	66.37	5.0
80.12	5.0	62.90	9.3
79.80	6.3	61.31	8.8
79.01	7.5	60.12	13.0
76.30	11.8	60.01	2.5
76.01	13.8	20.05	1.3
75.12	14.3	16.51	8.0
74.26	17.3		

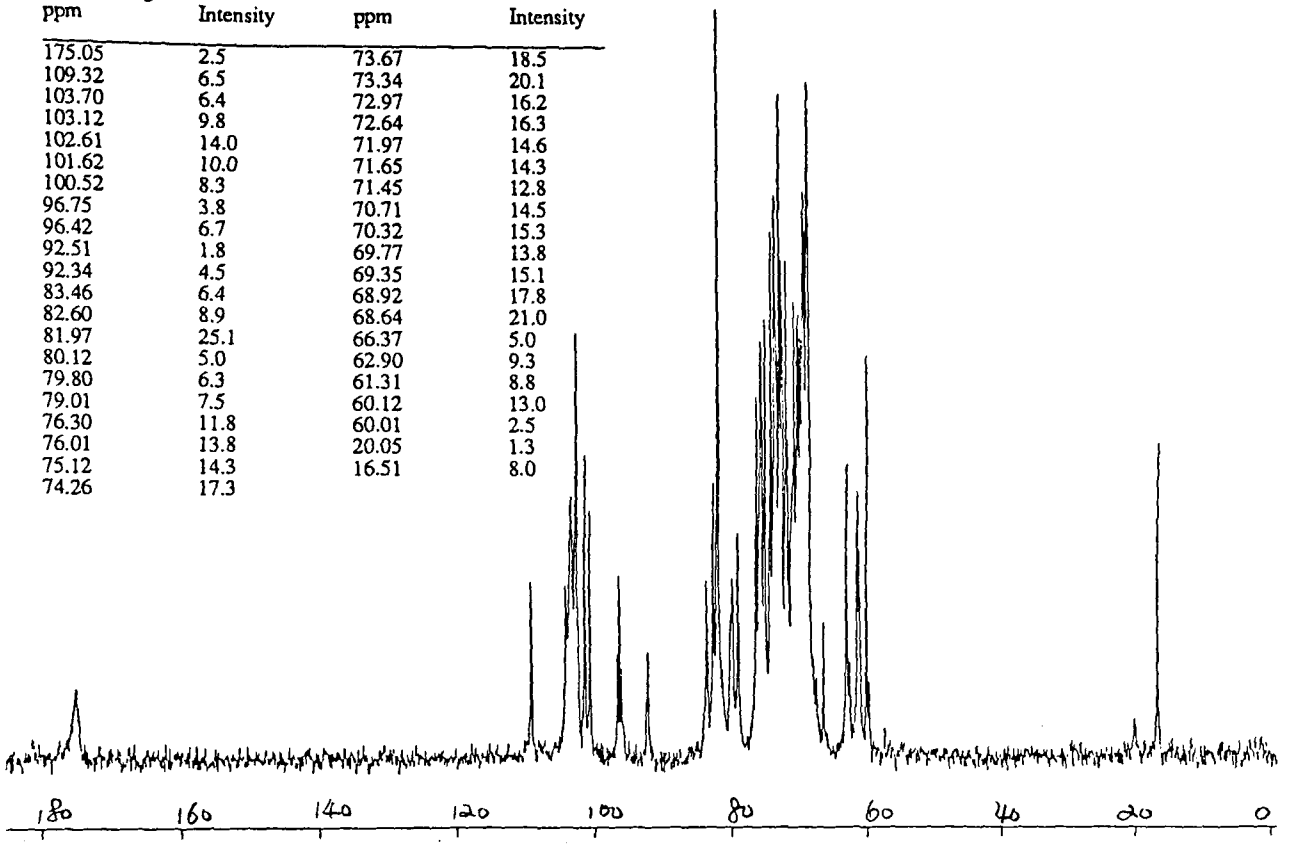

Fig. 7.4 ^{13}C NMR spectrum for *Leucaena collinsii subsp. zacapana* gum δ(ppm)

L. esculenta gum

ppm	Intensity
175.09	1.4
109.44	5.0
108.27	3.4
102.91	9.0
100.65	12.3
100.08	2.1
83.59	7.0
82.68	4.6
81.31	11.9
80.30	4.8
79.04	10.3
76.65	6.5
76.00	12.9
74.21	17.1
73.26	20.7
71.91	16.3
71.40	6.0
70.26	24.4
70.01	25.0
68.90	23.3
63.26	3.3
61.21	20.7
16.55	12.6

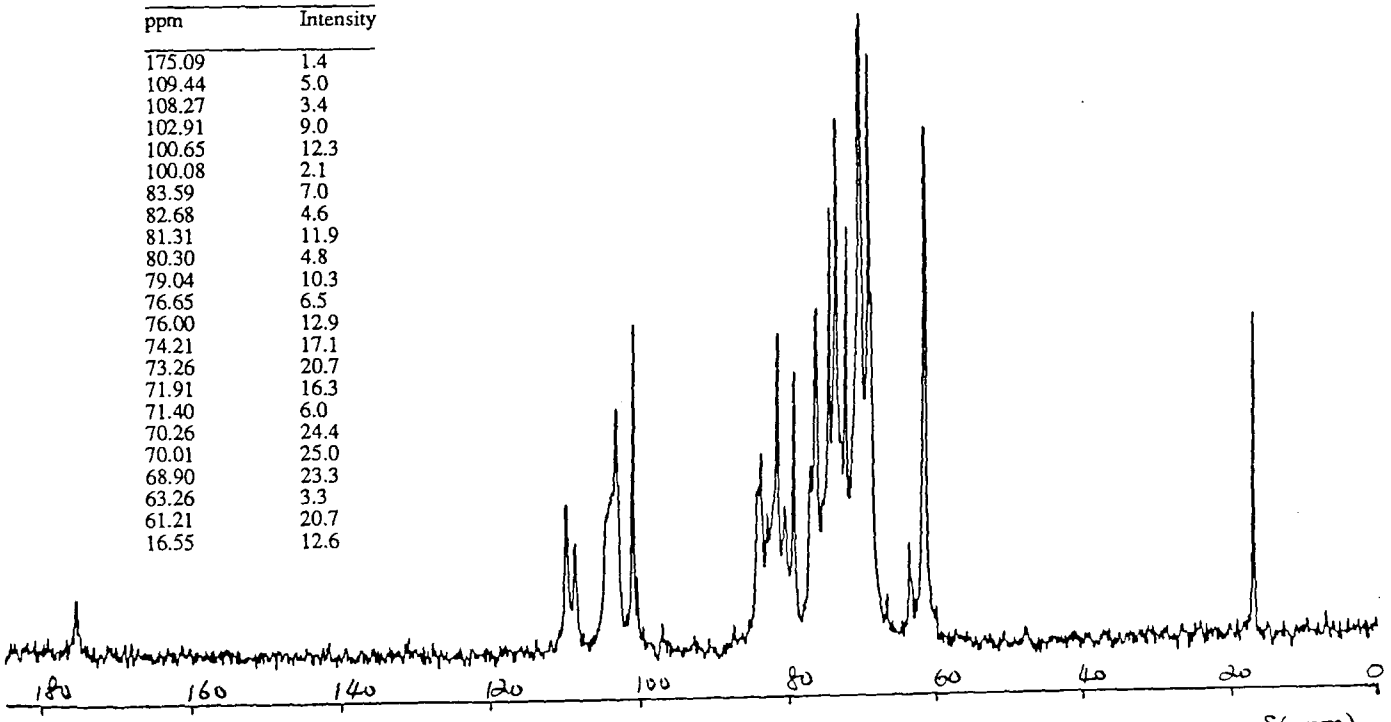

Fig. 7.5 ^{13}C NMR spectrum for *Leucaena esculenta subsp. esculenta* gum δ(ppm)

L. diversifolia gum

ppm	Intensity	ppm	Intensity
109.35	8.3	74.09	12.2
107.30	2.5	73.62	11.9
104.00	3.0	73.24	13.2
103.09	8.0	72.86	13.0
102.57	8.2	72.51	12.2
102.30	7.0	72.38	14.7
101.33	5.5	71.80	18.6
100.64	7.3	70.67	6.7
100.15	1.1	70.20	11.4
99.28	1.1	69.97	12.7
96.68	8.3	69.78	13.8
96.34	7.5	69.23	20.6
92.49	4.7	68.86	16.3
92.25	4.2	68.54	19.9
87.30	1.2	68.40	25.3
83.75	8.9	67.38	2.3
82.58	4.4	66.30	13.0
81.90	15.0	65.55	1.5
81.26	9.8	64.97	1.2
80.03	7.5	62.82	6.5
78.94	7.4	62.38	7.1
76.50	8.7	61.18	15.7
76.21	7.7	59.96	5.0
75.61	9.5	16.43	6.1
74.91	8.9		

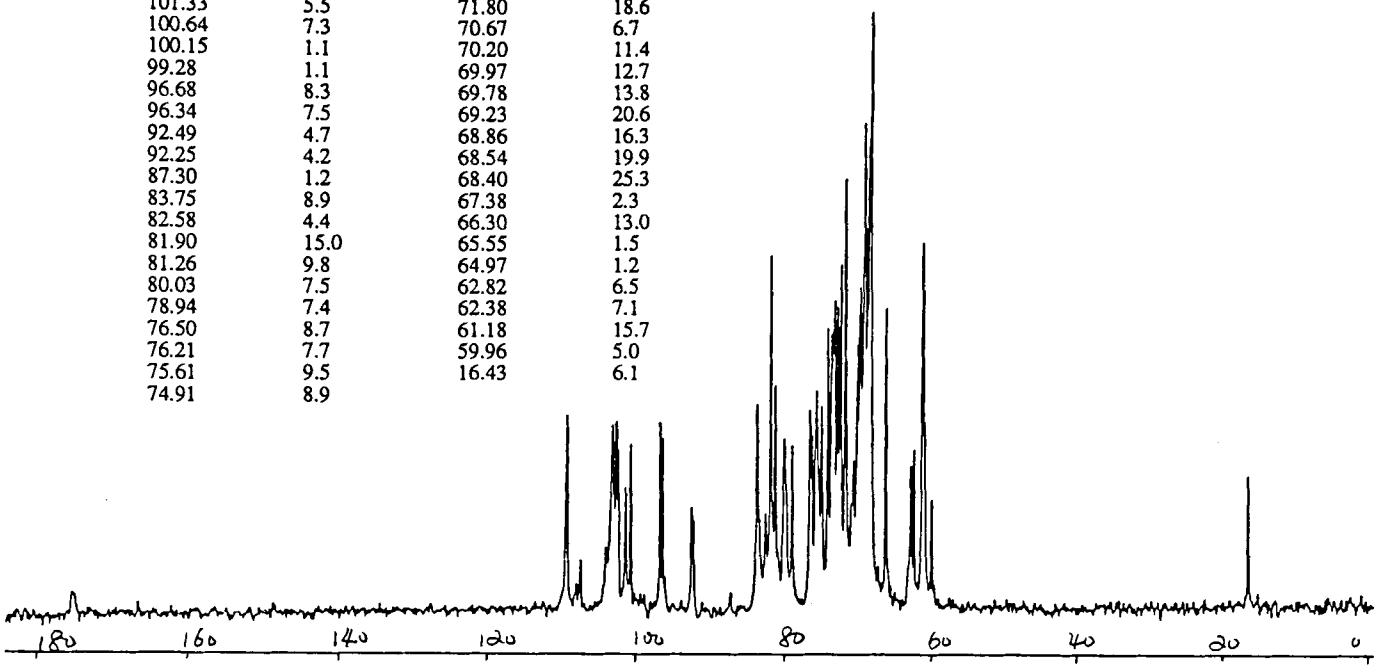

Fig. 7.6(a) ^{13}C NMR spectrum for *Leucaena diversifolia subsp. stenocarpa* gum δ(ppm)

L. diversifolia gum after dialysis

ppm	Intensity	ppm	Intensity
175.11	1.2	74.09	23.3
109.26	10.4	73.32	13.3
107.37	3.7	72.83	11.4
103.05	8.2	71.82	7.9
102.55	10.0	70.66	5.9
101.31	15.4	69.99	11.8
100.57	4.8	69.72	14.2
96.32	1.7	69.22	13.4
92.26	1.6	68.72	13.2
83.51	11.7	68.55	11.2
82.56	13.3	66.09	1.5
81.86	25.0	62.82	17.9
79.71	11.9	61.21	18.4
78.89	5.8	59.95	3.8
76.21	19.1	16.45	4.5
75.56	10.2		
74.99	9.3		

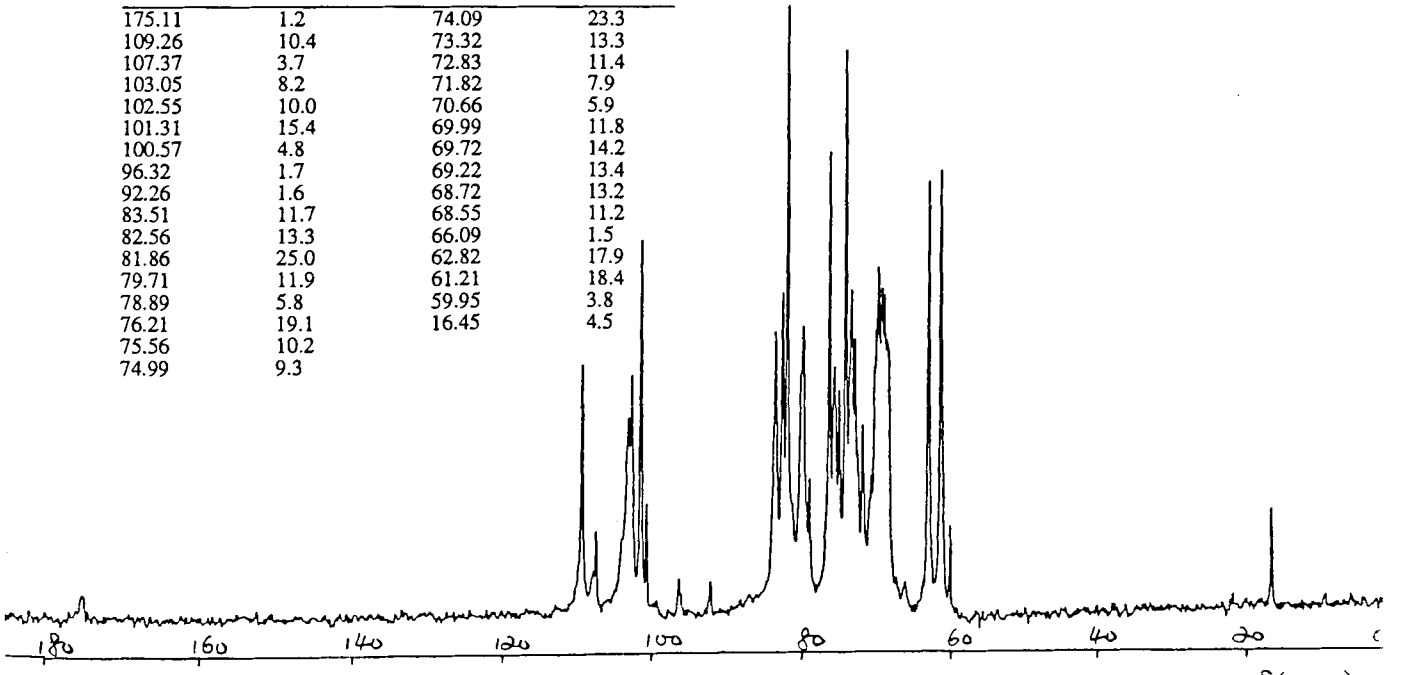

Fig. 7.6(b) ^{13}C NMR spectrum for the above gum after dialysis

The amounts of free sugars and the ratio of free Gal:Ara vary in those Central American *Leucaena* gums. Most have more galactose than arabinose, except for *Leucaena diversifolia* (Fig. 7.6(a)) and this reflects the structural differences shown by the *Leucaena* gums. Only *Leucaena esculenta* gum contains a considerable amount of internal α-L-Araf (108.3 ppm) and its spectrum (Fig. 7.5) is the closest to that of *A. senegal* (Fig. 3.1); as their analytical parameters are fairly similar, the presence of this gum would be difficult to detect if it became available in quantity to gum blenders as a cheap potential adulterant of gum arabic.

The unnamed *Leucaena sp.* (Fig. 7.2) has almost no arabinose involved in its main structure because only a very small amount of α-L-Araf (109.3 ppm) exists ; one possible structural feature may involve Rham 1→4 GlupA 1→6 or ? Gal 1→ because there are such sharp resonances for Rham (100.6 ppm and 16.4 ppm for its C_1 and C_6 respectively) and sharp 78.9 ppm for C_4 of (1→4) GlupA. It appears that this type of gum possesses a galactan core with major 1→6 linkages, because there is only a small 60.9 ppm signal contributed by free galactose C_6, indicating that almost all C_6 positions on galactose are involved in linkages.

Chapter 8
Studies of Combretum Gum

8.1 Introduction

The gum exuded by species of the *Combretaceae* has been collected regularly and offered for sale for many years. Species of the *Combretaceae* occur in the Sudan, in West Africa and in East Africa and their exudates are liable to be found in admixture with the gum from other genera (e.g. *Acacia* in parcels originating from these different markets).

The family *Combretaceae* contains two sub-families; one of these (*Combretoideae*) contains two tribes, of which one (*Combreteae*) contains three sub-tribes and these contain 16 genera in all. From the point of view of gum chemistry two of the three sub-tribes contain the important genera: the sub-tribe *Combretineae* contains the genus *Combretum* (which contains 200 spp.) and the sub-tribe *Terminaliinae* contains the genera *Anogeissus* (14 spp.) and *Terminalia* (150 spp.) (Anderson 1978b).

The genus *Combretum* Loefl., cosmopolitan in the tropics and sub-tropics but absent from Australia, is the largest and one of the most complex in the Family *Combretaceae* (order *Myrtales*). There are ca. 180 different Africa spp. and ca. 30 different Asian spp., which have been given more than 600 specific names by botanists over the years. Examples of the extensive synonymy that can arise, and a summary of the taxonomic classification of the Family *Combretaceae*, have been given (Anderson and Bell 1977). These taxonomic difficulties have arisen because the genus *Combretum* is a complex and heterogeneous population in which there appears to be a constant reshuffling of genes; a number of characters are found in nearly every combination. All that can be done is to give the "complexes" or "aggregates" of species the earliest, legitimate name available.

The Sahelian droughts in 1972-1974 and 1983-1985 led to serious shortages of gum arabic (*Acacia senegal* (L.)Willd.) but not of *Combretum* gums which became used extensively as adulterants. The resulting products were very unsatisfactory; *Combretum* gums have completely different characteristics and cannot be used as acceptable substitutes for gum arabic; moreover, *Combretum* gums have never been permitted under any international regulatory system; toxicological safety evaluations

have not been reported for the gum from even one of the many *Combretum* species. Nevertheless, *Combretum* gum, which is readily available at very low price throughout West Africa, has become used to an increasing extent and the availability of data that can be used to detect it has become important.

The gums from *Combretum* spp. have a wide variability in specific rotations and uronic acid contents (Anderson et al. 1986a; Anderson and Morrison 1990). The acidic units include not only D-GlupA and its 4-methyl ether, but also D-GalpA together with D-Gal, L-Ara and L-Rha in all species and D-Xyl and D-Man in some. The most interesting feature is the location of the rhamnose and uronic acid residues; in *C. hartmannianum* gum the rhamnose and uronic acid occupy chain-terminal positions (Anderson and Bell 1976) i.e would tend to be available on the periphery of the gum molecule, as occurs in the *Acacia* group; whereas in *C. leonense* gum, however, very few of the uronic acid and rhamnose residues were present as end group (Aspinall and Bhavanadan 1965) and recent work has shown that this is also a structural feature of *C. nigricans* gum (Anderson et al. 1991).

8.2 Analytical Studies of Combretum nigricans Gum

Combretum nigricans gum is the major source of commercial "gum combretum" which is widely available in West African markets. Although not permitted in foodstuffs by the regulatory authorities it has been used as an adulterant of gum arabic (*Acacia senegal*).

Combretum nigricans Lepr.ex Guill. et Perr. occurs widely as a typically variable aggregate throughout tropical West Africa, particularly in northern Nigeria, Mali and Niger. It exudes gum copiously, and contributes substantially to the tonnages available in these countries. Gum combretum varies greatly in appearance, ranging from large, dark brown or black glossy masses, through smaller circular or oval-shaped reddish-brown lumps to even smaller pieces that are often kidney-shaped and very pale yellow in colour. Usually one end of the nodules is strongly pigmented with an intense carmine-red substance at the point where the gum was attached to the tree bark. Poorer grades in addition do not dissolve completely but form variable proportions of gel; regardless of the pale colour of some gum nodules, combretum gum solutions are invariably dark reddish-brown in colour. Combretum gum pieces are always smooth and opaque in appearance; this is characteristically different from the appearance of good quality gum arabic which is more crystalline in external appearance with prominent, characteristic, rough surface markings. Gum combretum

also usually has a distinct acetous odour, arising from its acetyl content. Additionally, it easily forms "blocked "gum by self-adhesion during storage.

8.2.1 Origin of Gum Samples

The collection of six small samples (#1 to #6) of gum, each from separate *Combretum nigricans* trees growing at Sadore, Niger was arranged by Dr. R.J. van den Beldt, Principal Agroforester ar ICRISAT, Niamey. Two large gum samples from *Combretum nigricans* Lepr.ex Guill. et Perr. (samples #7 and #8) were provided by Mr. Oseni, Department of Forests, Ibadan, Nigeria. One spray-dried commercial sample (#9) of "gum combretum" from West Africa was included for comparison purposes. All natural gum samples were clear and pale yellow in colour, although their solutions varied in colour from yellow to reddish-brown.

8.2.2 Results and Discussion

The physico-chemical parameters and amino acid compositions for *Combretum nigricans* gum samples #1 to #9 are shown in Table 8.1. For the first time, however, it has been possible to ascertain the extent of the resulting variability in gum chemistry parameters for the *Combretum nigricans* aggregate in terms of the analytical differences between those single tree samples collected by hand. The extent of the variability is comparable with that recorded for *Combretum leonense* in the earliest study of this type (Anderson et al. 1959). The inter-tree variability for *Combretum* species is much greater than has been encountered in gum samples from *Acacia senegal* (L.) Willd. (Anderson et al. 1990) and from other *Acacia* species for which such studies have been made, e.g. for *Acacia nilotica* gum (Anderson and Karamalla 1966) and *Acacia laeta* gum (Anderson and Smith 1967).

The analytical data in Table 8.1 show that there are relatively wide variations in the nitrogen content (0.15-0.50%); specific rotation (-28° to -85°) and Ara:Gal ratio (1.48 to 2.36) of the single tree samples.From comparisons with other reported *Combretum* species (Anderson et al. 1986a; Anderson and Morrison 1990) the features characteristic of *Combretum nigricans* gum are: a relatively strong negative specific rotation (the mean specific rotation value of these single tree samples is -53°, which is very close to the value (-55°) for commercial sample #9); a low rhamnose content (5-9%); more arabinose than galactose; a relatively high acetyl content (1.3-4.2%); and a low uronic acid content (8-14%) in which the amount of GalpA is more than that of GlupA. In contrast, some *Combretum* species contain more GlupA than GalpA.

Table 8.1 Analytical data for *Combretum nigricans* gum samples

Items	No. of gum samples #1	#2	#3	#4	#5	#6	#7	#8	#9
H_2O%	12.0	11.0	10.0	9.2	9.2	10.4	13.5	10.8	9.4
Solubility%	100	100	100	100	100	100	80	90	100
Ash%	2.7	1.7	2.1	1.4	1.3	2.0	2.4	2.3	2.3
N%	0.15	0.50	0.39	0.44	0.24	0.33	0.31	0.22	0.41
NCF	6.47	6.41	6.23	6.47	6.34	6.18	6.66	6.25	6.22
Protein%	0.97	3.21	2.43	2.85	1.52	2.04	2.06	1.37	2.55
Methoxyl%	0.22	0.20	0.18	0.15	0.17	0.18	0.20	0.21	0.15
Acetyl%	3.5	2.0	2.1	3.0	1.3	3.8	3.6	4.2	3.5
$[\alpha]_D$	-28°	-61°	-39°	-85°	-64°	-48°	-46°	-53°	-55°
[η]ml/g	39	33	23	19	23	33	40	35	29
E.Wt[a]	1260	1710	1420	2340	1920	1480	1300	1480	1850
U.A.A	14	10	12	8	9	12	14	12	10
Tannin%	0.10	0.15	0.11	0.23	0.08	0.09	0.88	0.19	0.50
Sugar composition after hydrolysis%									
4-O-MGUA[b]	1	1	1	1	1	1	1	1	1
GlupA	2	1	1	1	1	1	5	4	3
GalpA	11	8	10	6	7	10	8	7	6
Gal	31	28	25	26	26	24	29	35	32
Ara	46	55	54	61	59	55	49	48	50
Rha	9	7	9	5	7	9	8	5	8
Aminoacid compositions (per 1000 residues)									
Ala	104	94	103	80	96	98	75	92	85
Arg	18	16	20	17	23	25	16	24	17
Asp	99	105	112	102	117	122	140	111	92
Cys	34	0	9	0	71	41	26	5	8
Glu	110	115	102	119	72	70	57	78	78
Gly	105	103	135	102	127	129	79	155	121
His	32	38	29	31	27	31	22	22	29
Hyp	47	0	0	0	5	0	68	65	0
Ile	39	30	38	29	38	40	22	33	28
Leu	49	45	58	42	53	58	37	50	37
Lys	28	28	36	25	35	34	24	34	51
Met	10	10	9	6	8	10	7	6	6
Phe	39	36	22	28	40	27	33	29	27
Pro	64	177	92	224	47	69	160	70	205
Ser	69	68	80	61	80	84	72	77	87
Thr	66	55	69	56	67	75	86	60	52
Tyr	36	38	35	38	48	38	40	46	40
Val	51	42	51	40	46	49	36	43	37
NCF	6.47	6.41	6.23	6.47	6.34	6.18	6.66	6.25	6.22

a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).

Table 8.1 also shows that *Combretum nigricans* gum has a characteristic amino acid composition; Gly, Asp, Ala and Glu are the major amino acids with a very low proportion or even absence of Hyp, which is the major amino acid in gum arabic (*Acacia senegal*). This confirms previous indications (Anderson and Morrison 1990) that *Combretum* gums differ widely from *A. senegal* gum in terms of their amino acid composition.

8.3 Structural Studies of Combretum nigricans Gum

8.3.1 Introduction

The genus *Combretum* produces gum exudates that are more viscous, of higher molecular weight and more acidic than those of the genus *Acacia.* Gum exudates from *Combretum* species consist of both D-GlupA and D-GalpA, and D-Gal, L-Ara and L-Rha in all cases; D-Xyl and D-Man also occur in some species such as *C. hartmannianum* (Anderson and Bell 1976), *C. apiculatum* and *C. obovatum* gums (Anderson et al. 1986).

Combretum nigricans gum contains GalpA as well as GlupA, has low nitrogen and rhamnose contents, and has a very high arabinose content. However, none of these superficial analytical differences explain the great differences in functionality between gum arabic and *Combretum nigricans* gum. Some structural differences were revealed by a methylation analysis of *Combretum nigricans* gum (Anderson et al. 1991); *C. nigricans* gum contains a great proportion of internal (1→2)linked rhamnose and even some 1,2,4 linked rhamnose as branch points, and both (1→4) linked GlupA and GalpA. Thus its structure differs greatly from the long-established structural features of gum arabic; in which all rhamnose residues are chain-terminal and joined to glucuronic acid with these two sugars occupying peripheral sites in the globular, highly branched, gum macromolecules. On acidic degradation, degraded gum arabic essentially contains only Gal and GlupA; all Rha and almost all Ara units are eliminated. On periodate oxidation, the Ara and Gal units are largely resistant but all of the Rha and most of the GlupA are eliminated.

Combretum nigricans gum behaves in a very different way. Both acidic and periodate degradations lead to the isolation of degraded polysaccharides, for which analytical data, including their amino acid compositions and ^{13}C NMR spectra, are presented in this section and the specific structural features are deduced.

8.3.2 Material and Experiments

Sequential Smith-degradations: 50g *Combretum nigricans* gum (sample #8) was dissolved in 1250ml distilled water overnight; 1250ml 0.25M $NaIO_4$ solution was added, giving a 2% gum solution for periodate oxidation. The procedures used were as described in Chapter 2. Smith-degradation products SD1, SD2, SD3 and SD4 were obtained.

Partial acid hydrolysis; 10g *Combretum nigricans* gum in 500ml 0.005M H_2SO_4 was for 96 hours under reflux in a boiling water bath, followed by the procedures described in Chapter 2. A degraded gum D_{acid} was obtained.

^{13}C NMR spectra of the original *C. nigricans* gum and all of its degraded products were obtained.

8.3.3 Results and Discussion

Table 8.2 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 8.1) for *C. nigricans* gum

Chemical shift (ppm)	C_1 of
109.3	α-L-Araf(1→
108.2	→α-L-Araf(1→
107.4-106.8	→3)α-L-Araf(1→3)Araf(1→
(104)	→α-L-Arap(1→ (with 66.4 ppm from C_5)
103.3	→β-D-Gal(1→) and β-D-Gal(1→ or →β-D-GlupA(1→ and β-D-GlupA(1→
101.4	→3)β-L-Araf(1→ (with 62.9 ppm from C_5)
100.7	α-L-Rham(1→ →β-D-GalpA(1→ and β-D-GalpA(1→
20.0	carbon of acetyl (-OCO-$\underline{C}H_3$)

Linkage	C_1	C_2	C_3	C_4	C_5	C_6
α-L-Araf(1→	109.2	80.3	76.5	83.8	61.1	
→3)α-L-Araf(1→	108.2	81.2	83.8	83.8	61.1	
→5)α-L-Araf(1→	108.2	81.2	76.5	83.8	68.5	
→2)α-L-Araf(1→	108.2	86.9	76.5	83.8	61.1	
	107.4		75.8			
	106.8					
β-D-Gal(1→	103.3	70.3	72.6	68.9	75.0	61.1
→4)β-D-Gal(1→	103.3	70.3	72.6	73.9	73.3	61.1
→6)β-D-Gal(1→	103.3	70.3	72.6	68.9	73.9	70.3
→4,6)β-D-Gal(1→	103.3	70.3	72.6	73.9	73.9	70.3

Combretum nigricans gum

ppm	Intensity
171.11	1.1
109.32	8.1
108.18	3.9
107.36	4.8
106.76	7.7
103.27	6.7
101.37	2.6
100.72	1.7
96.71	1.1
86.85	2.0
83.82	14.5
81.24	21.5
80.41	7.6
76.49	17.1
75.79	10.4
74.99	5.5
73.93	9.4
73.32	9.6
72.56	6.1
71.85	5.1
70.30	8.9
68.88	7.0
68.45	7.2
66.37	4.7
62.86	3.6
61.14	25.0
54.38	2.4
48.58	2.2
35.99	2.7
20.04	1.0
16.45	3.2

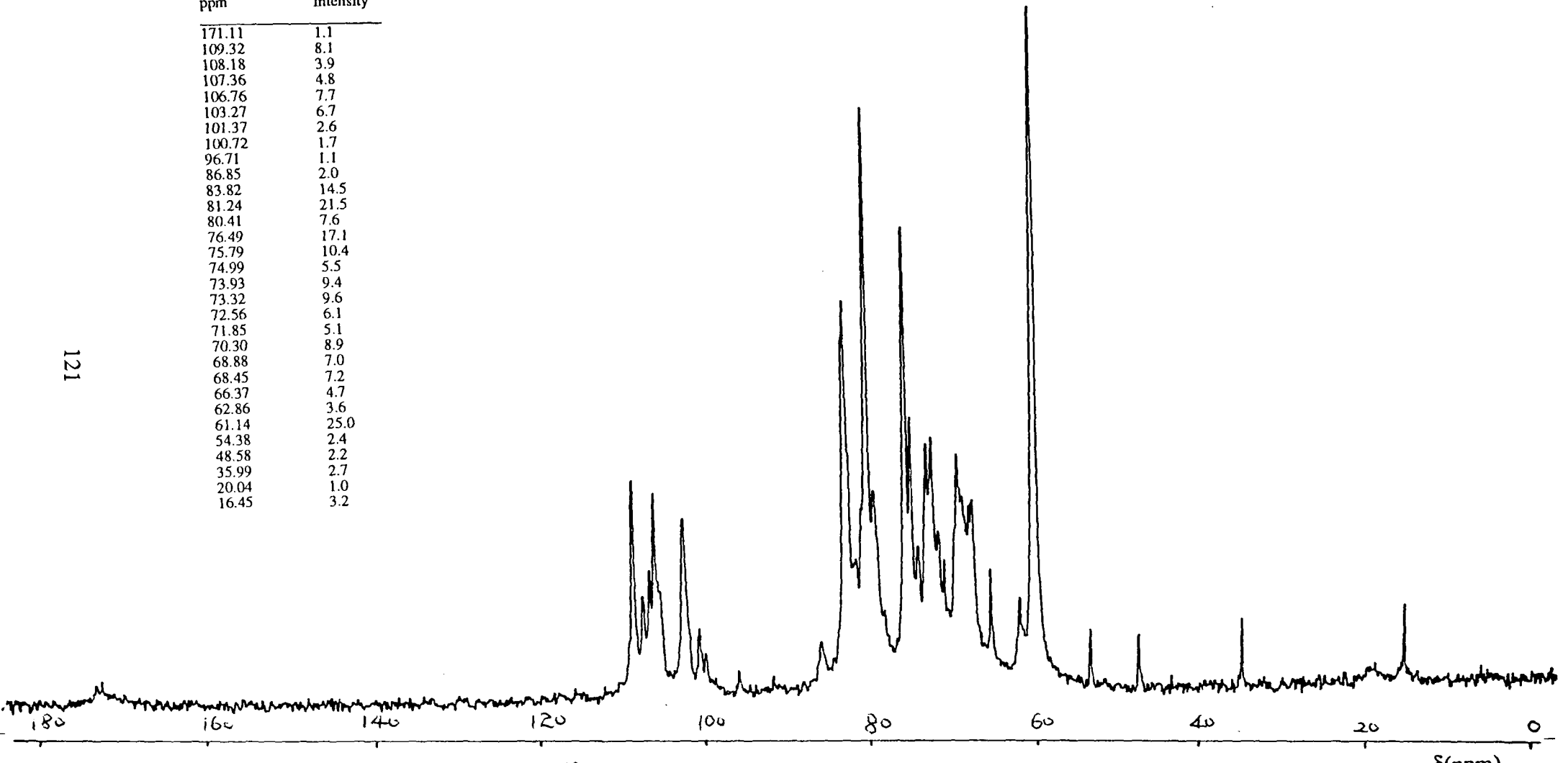

Fig. 8.1 ^{13}C NMR spectrum for *Combretum nigricans* gum

Table 8.3 Analytical data for the degraded products from *C. nigricans* gum

Amino Acids	C. *nigricans*	The degraded products SD1	SD2	SD3	SD4	D_{acid}
Ala	92	95	99	98	103	105
Arg	24	17	9	18	14	17
Asp	111	118	149	134	145	38
Cys	5	0	0	0	0	2
Glu	78	65	82	83	131	105
Gly	155	129	125	137	134	135
His	22	21	21	15	16	32
Hyp	65	81	45	51	29	53
Ile	33	42	43	39	34	42
Leu	50	54	60	55	54	57
Lys	34	29	34	32	30	36
Met	6	8	4	11	1	15
Phe	29	36	35	31	29	34
Pro	70	63	48	47	73	47
Ser	77	82	100	98	87	109
Thr	60	70	69	67	67	79
Tyr	46	41	27	34	4	38
Val	43	49	50	50	49	56
NCF	6.25	6.47	6.41	6.43	6.46	6.30
Yield(g)	50.0	21.5	12.5	8.0	2.0	1.0*
N%	0.41	0.14	0.16	0.20	0.37	0.25
Protein%	2.56	0.91	1.03	1.29	2.39	1.58
Protein mg	1280	196	129	103	48	2.5*
$[\alpha]_D$	-55°	-27°	-22°	-16°	-8°	+73°
[η]ml/g	35	18	10	8	6	4
U.A.A	10	15	17	21	25	33
Sugar composition after hydrolysis%						
GlupA	5	7	8	12	15	12
GalpA	7	8	9	9	10	21
Gal	35	34	32	37	32	25
Ara	48	43	35	23	20	6
Rha	5	8	16	19	24	36

* Starting from 10 g gum or 256 mg protein.

^{13}C NMR spectrum of *C. nigricans* gum (Fig. 8.1) shows its characteristic "fingerprint". The major sugar assignments are listed in Table 8.2. That arabinose is the major monosaccharide constituent (ca. 50%) in *C. nigricans* gum has been confirmed by its ^{13}C NMR spectrum. The main chemical shifts (83.8, 81.2, 76.5 and 61.1 ppm) in the spectrum represent arabinose carbons (Table 8.2); more precisely, most of the arabinose is in the α-L-Araf form. This differs greatly from *Acacia seyal* or *Acacia sieberana* gums, which mainly contain β-L-Arap although the Ara:Gal sugar ratios are nearly the same in *C. nigricans* and *A. seyal* gums. The different

arabinose residue forms and linkages lead to the great differences in the specific rotation values between *C. nigricans* gum (ca. -55°) and *A. seyal* gum (ca. +62°) or *A. sieberana* gum (ca. +114°). This reveals that α-L-Araf makes a contribution to the negative specific rotation of the gum, whereas β-L-Arap leads to a positive rotation.

The most interesting spectral feature is the three chemical shifts (54.4, 48.6 and 36.0 ppm) of nearly the same intensity which are unique and can be used to identify *C. nigricans* gum. And these carbons are located at peripheral positions in the *C. nigricans* gum structure because they are not found in the spectra of degraded products. This may suggest that these carbons may be involved in other linkages to the α-L-Araf in *C. nigricans* gum.

Table 8.3 shows the physico-chemical parameters of the degraded products. The nitrogen contents are enriched considerably in the core product (from 0.14% in SD1 to 0.37% in SD4) during sequential Smith-degradations, but there are no great differences in the amino acid compositions except for Glu increasing and Tyr decreasing. In the acid-degraded gum D_{acid}, Asp and Pro are low but Glu and Ser are high in comparison with the amino acid composition of the original *C. nigricans* gum. Specific rotation values decrease from -53° to -8° in SD4 with the arabinose content decreasing progressively (Table 8.3), while the rhamnose and uronic acid contents are enriched during Smith-degradation. This shows that the major uronic acid and rhamnose units are located in internal positions in *C. nigricans* gum, very different from their peripheral, chain-terminal position in gum arabic (*A. senegal*).

The sugar composition of D_{acid} shows that the major sugar constituents are rhamnose (36%) and uronic acid (33%), with the arabinose almost completely eliminated (from 48% in the original gum to 6% in D_{acid}). The specific rotation changed greatly from -53° to +73°. Figs. 8.2, 8.3 and 8.4 are the ^{13}C NMR spectra for the sequential Smith-degradation products SD1, SD2 and SD3 respectively. Fig. 8.2 shows that the signals between 106 and 109 ppm decreased distinctively, indicating that α-L-Araf at peripheral positions has been eliminated to some extent; and the Ara:Gal sugar ratio changes from 1.37 in the original gum to 1.08 in SD1. Fig. 8.3 shows that the rhamnose residues start to become relatively more peripheral at this stage of the degradation process, indicated by the much higher $-CH_3$ carbon signal of rhamnose (16.5 ppm). α-L-Araf and β-D-Gal are being eliminated continuously, but the β-L-Arap contents shown at 96.7 ~97.8 ppm in SD2 (Fig. 8.2), are increasing greatly. This indicates that the α-L-Araf residues are mainly located at peripheral positions or are chain-terminal in the *C. nigricans* gum structure, whereas small

C. nigricans gum Smith-degradation product SD1

ppm	Intensity
109.23	3.2
107.39	2.2
103.29	7.1
101.60	2.3
96.96	1.8
88.17	2.8
85.69	1.5
83.82	6.4
81.27	6.7
80.07	4.0
78.95	4.8
76.51	6.8
74.81	5.6
73.51	7.1
70.69	7.9
68.53	5.9
67.79	4.6
63.02	3.9
61.95	7.2
61.27	25.0
58.61	2.8
16.85	1.9

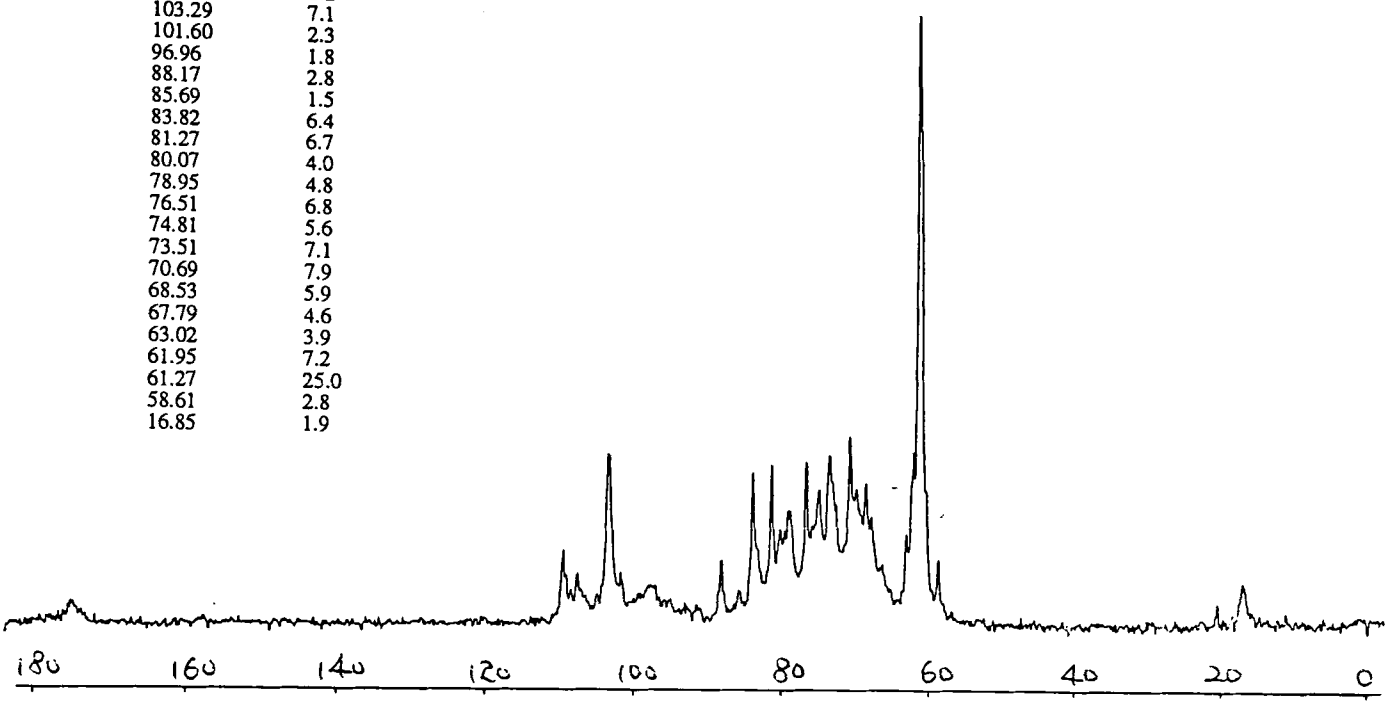

Fig. 8.2 ^{13}C NMR spectrum for degraded *C. nigricans* gum (SD1) δ(ppm)

C. nigricans gum Smith-degradation product SD2

ppm	Intensity	ppm	Intensity
177.99	1.3	75.00	8.0
176.77	2.4	73.62	6.1
174.89	1.5	72.58	8.6
109.15	3.6	71.48	7.8
107.03	1.2	70.64	11.2
103.20	10.5	69.82	10.3
100.37	1.3	68.56	11.7
97.84	3.0	67.74	5.8
96.75	3.7	65.34	1.7
95.75	1.8	62.66	10.2
83.83	5.5	62.28	10.2
81.21	7.5	61.26	25.1
78.64	4.8	60.94	20.8
77.10	10.8	16.66	16.2
76.52	16.1	15.86	1.9

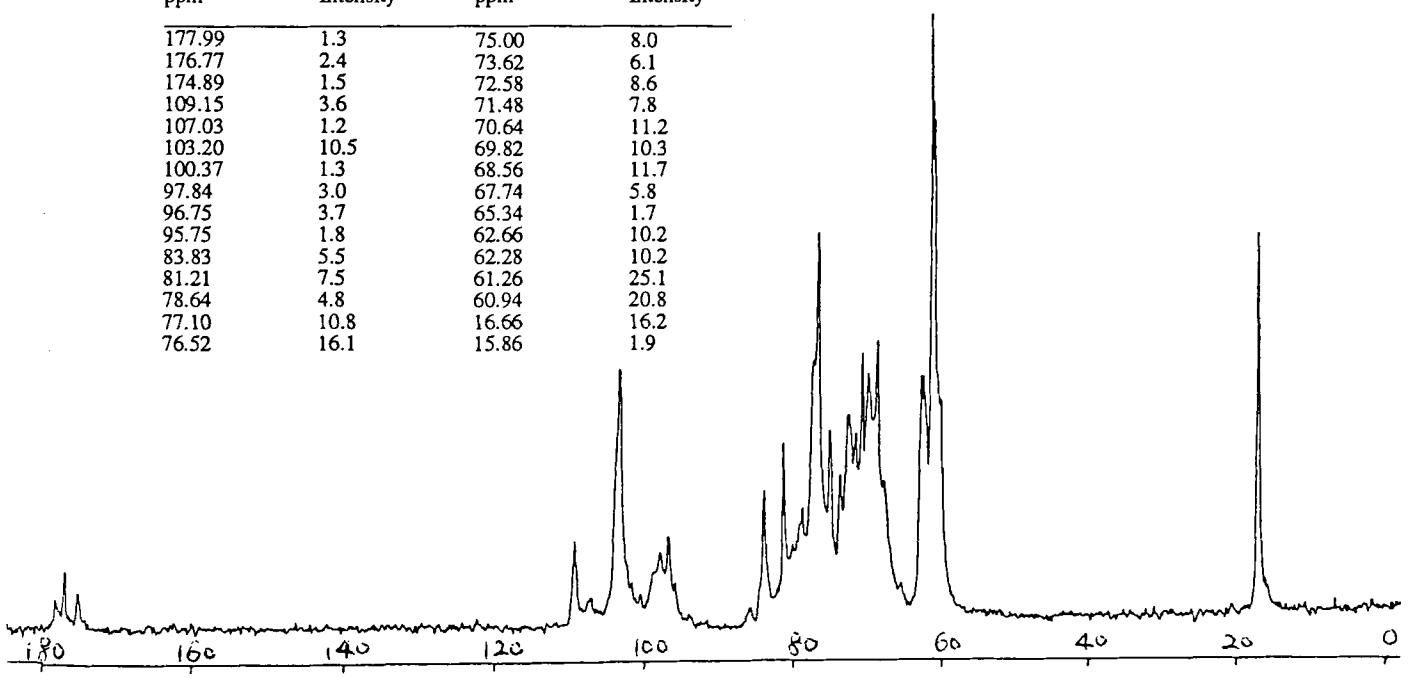

Fig. 8.3 ^{13}C NMR spectrum for degraded *C. nigricans* gum (SD2) δ(ppm)

C. nigricans gum Smith-degradation product SD3

ppm	Intensity
177.93	2.2
176.68	3.1
102.86	13.9
100.34	10.5
96.71	12.0
91.52	1.3
80.21	5.8
78.01	11.3
77.39	20.0
76.53	15.53
75.88	7.9
75.01	5.3
72.09	17.2
71.52	3.6
70.64	3.1
69.78	12.0
68.77	13.6
67.29	4.7
65.36	20.1
62.68	24.7
62.25	25.1
60.92	22.6
60.46	21.5
16.78	8.5
16.56	16.3
15.77	10.5

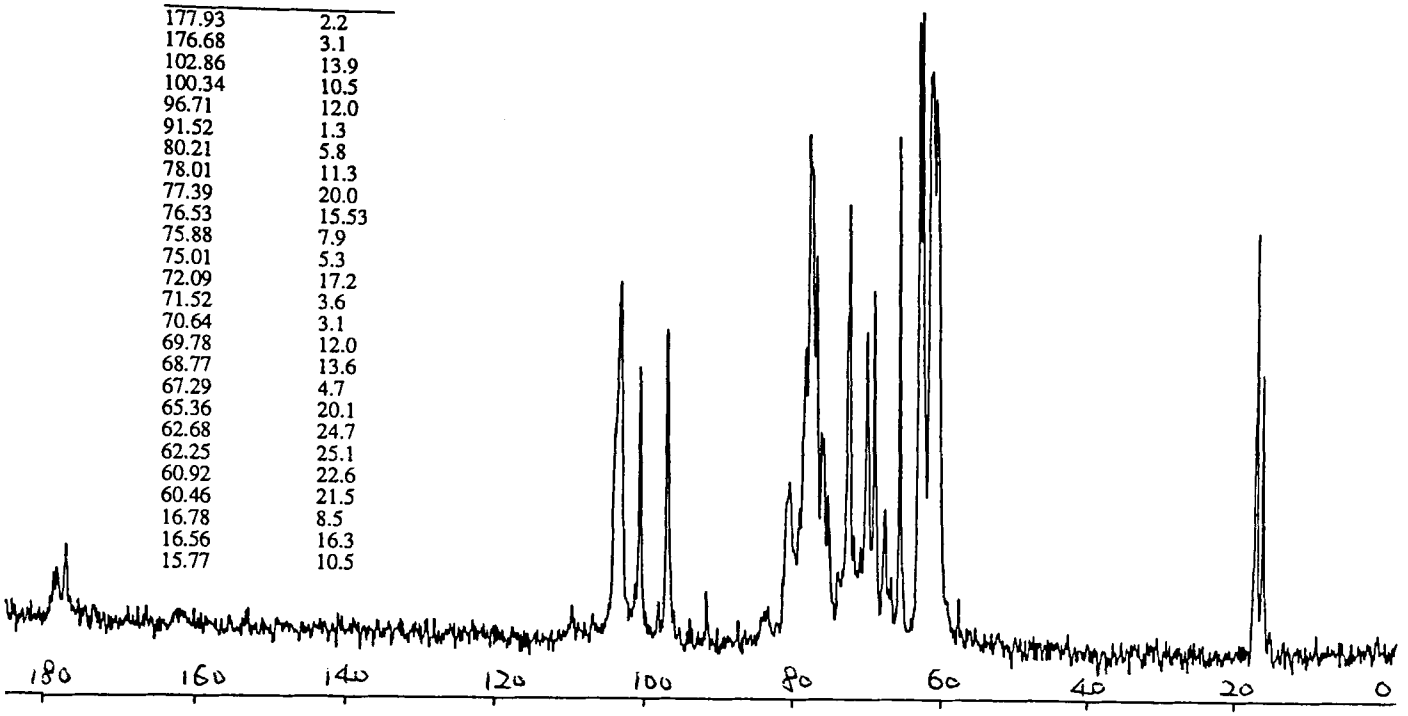

Fig. 8.4 ^{13}C NMR spectrum for degraded *C. nigricans* gum (SD3) δ(ppm)

C. nigricans gum (D_{acid})

ppm	Intensity
172.44	4.7
104.20	2.5
103.67	2.3
100.78	3.9
99.13	16.1
98.02	8.5
97.74	14.8
93.67	3.0
91.63	7.7
79.78	3.1
78.83	3.4
77.80	15.5
77.21	8.1
76.34	12.5
75.67	4.1
75.30	4.0
72.89	4.3
72.22	5.1
71.78	11.6
71.53	21.7
71.12	8.1
70.03	19.7
69.44	27.7
69.12	27.1
68.63	14.6
68.55	11.8
67.56	20.4
16.64	11.7
16.43	18.5

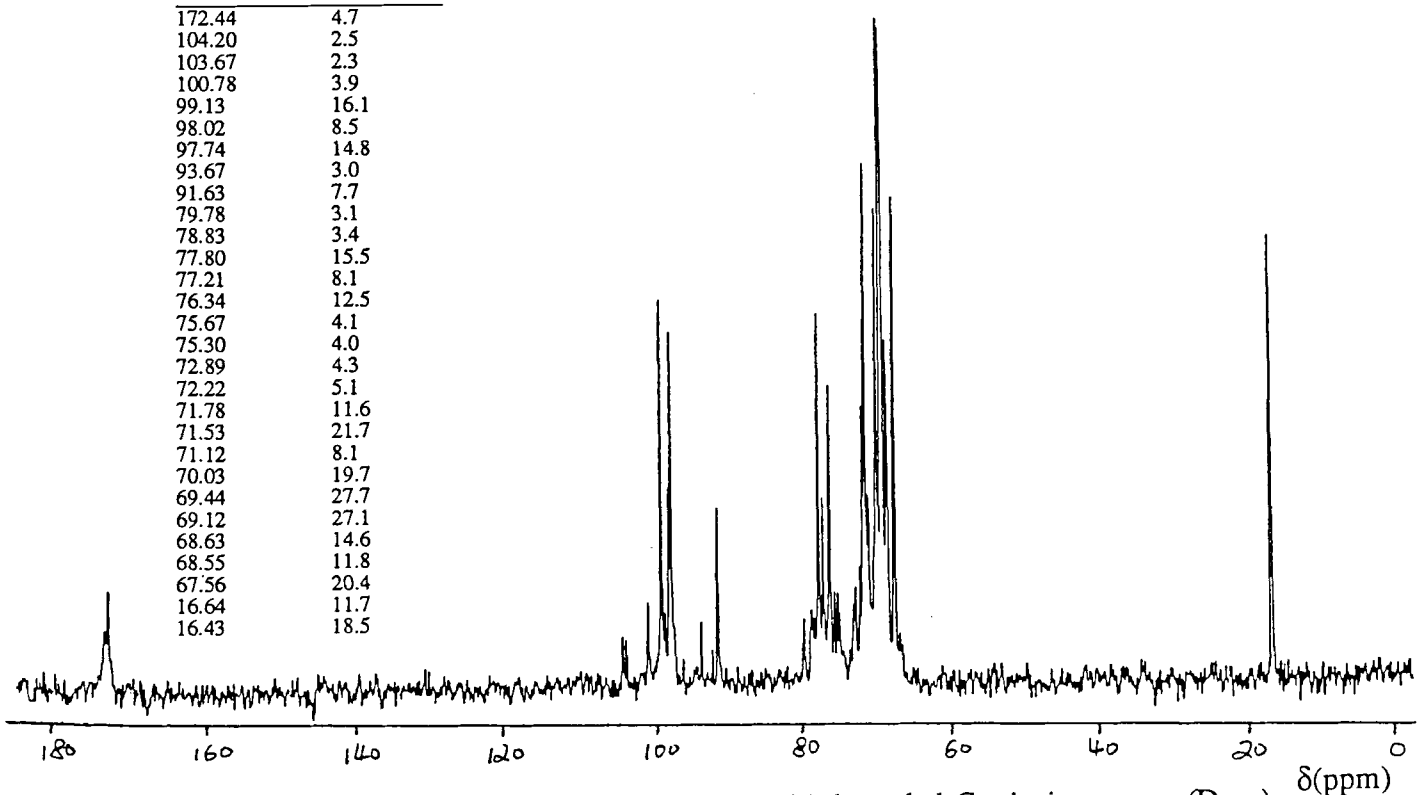

Fig. 8.5 ^{13}C NMR spectrum for partial acid-degraded *C. nigricans* gum (D_{acid})

proportions of β-L-Arap are located in internal positions. Furthermore, Fig. 8.3 shows a most interesting observation viz. that there are three L-Rha C_6 resonances at 16.8, 16.6 and 15.8 ppm respectively. This suggests that at least three different internal environments for rhamnose exist in *C. nigricans* gum. There is no α-L-Araf present in SD3; and β-L-Arap (96.7 ppm) is the main form of arabinose at this stage of the degradation, being almost entirely (1→2) or (1→3) links because of the very great intensities of the 62.3 and 62.7 ppm resonances from C_5 of β-L-Arap) (Fig. 8.3).

Table 8.4 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 8.5) for acid-degraded *C. nigricans* gum

Chemical shift (ppm)	C_1 of
104.2	α-L-Arap(1→ (with 67.6 ppm from C_5)
103.7	→β-D-GlupA(1→ and β-D-GlupA(1→
100.8	α-L-Rham(1→
99.1	→α-D-Gal
98.0	→2,4,α-L-Rha(1→
97.7	→2α-L-Rha(1→
93.7	reducing unit of →2β-L-Rha
91.6	reducing unit of →2α-L-Rha

Table 8.4 lists the assignments of the sugar constituents in the partial acid-degraded *C. nigricans* gum indicated by its ^{13}C NMR spectrum (Fig. 8.5). The α and β anomeric carbons in reducing L-Rha units with C_2 links are shown at 91.6 and 93.7 ppm respectively (Hoffman et al. 1986), which indicates that L-Rha in *C. nigricans* gum is internally linked. Moreover, the chemical shifts at 16.6 and 16.4 ppm show that the rhamnose in the acid-degraded gum has at least two different environments. Because there is only 6% arabinose in D_{acid}, the 97.7 ppm chemical shift with 14.8 intensity must be attibuted to 1,2,4 linked rhamnose C_1 (Vinogradov et al. 1991). Fig 8.5 also shows that internal α-D-Gal residues exist at 99.1 ppm (Kol et al. 1991) and that these are mainly (1→6) linked, because no 61.1 ppm chemical shift indicates that C_6 of galactose is involved in linkages. This differs greatly from the core structure of *A. senegal* gum, based mainly on (1→3) linked β-D-Gal.

The major features of *C. nigricans* gum are, therefore, (a) α-L-Araf, the major arabinose form, is peripheral together with β-D-Gal; (b) significant proportions of rhamnose (1,2 and 1,2,4 links) and uronic acids are not peripheral, but are involved in the inner core of the branched molecular structure; (c) α-D-Gal with (1→6) links and

β-L-Arap are also located in inner positions of the structure. The structures of *C. nigricans* gum and gum arabic are, therefore, fundamentally different, and this is reflected in their different functionality. An earlier methylation analysis (Anderson et al. 1991) showed that small proportions of rhamnose and uronic acid in *C. nigricans* gum were present as end groups, but that most of the rhamnose and uronic acids occupied intra-chain positions within the branched core of the complex gum molecules. Examination of the aldobiuronic acids isolated showed that at least some of the rhamnose and galacturonic acid were directly linked to each other.

The complex nature of the genus *C. nigricans* can now be illustrated by comparisons of the present conclusions with those of previous studies of *C. leonense* gum (Aspinall and Bhavanadan 1965) and *C. hartmannianum* gum (Anderson and Bell 1976). *C. nigricans* has features in common with *C. leonense* but these species appear to differ from *C. hartmannianum*. Thus, *C. nigricans* and *C. leonense* gums contain the same aldobiuronic acids and the majority of their uronic acids are intra-chain. But *C. hartmannianum* has galacturonic acid linked with mannose, not to rhamnose, and all of its uronic acids and rhamnose are present as end-groups. All three gums have large proportions of peripheral arabinose; this is largely in the pyranose form in *C. leonense* and *C. hartmannianum* gums, but, in contrast, is largely in the furanose form in *C. nigricans* gum.

Chapter 9
Analytical Studies of Seventeen Gum Exudates

Natural products such as gum arabic, tragacanth and karaya have long histories of safe use as pharmaceutical substances and as food additives, functioning as emulsifiers, emulsion stabilisers and viscosity modifiers. The availability of the natural gums enables food processors to meet the demands of a rapidly expanding segment of the market for "all-natural" products, in which synthetic or chemically modified additives are not acceptable. It has long been recognized and reiterated by WHO that naturalness does not assure safety (WHO 1978; 1987). Accordingly, the gums, in common with other additives, have been subjected to the appropriate, mandatory, toxicological tests. The nature and extent of such tests, involving at least many hundreds of animals, are also not widely known; a cost of at least a million pounds over a period of up to 10 years may be involved, with the regulatory committees thereafter taking up to 2 years to review all the evidence and announce their verdict (Anderson 1988). Strictly, no gum exudates can be regarded as permitted food additives without being subjected to appropriate food safety tests. Therefore a wide range of thousands of gum exudates from *Prosopis, Combretum, Albizia, Leucaena,* non-permitted *Acacia spp.*, and many other genera may have technological applications, but are not permitted for use in foodstuffs. In all cases some measure of analytical control is necessary and characterizations of the non-permitted gum exudates are desirable to provide the necessary data to help ensure that non-permitted gums are not used as adulterants of the permitted gum exudates.

9.1 The Composition and Properties of Ten Gum Exudates (Leguminosae) of American Origin

The Family Leguminosae comprises some 17500 species in about 670-700 genera with a world-wide distribution (Allen and Allen 1981). Fortunately only a very small proportion of these yield gums in commercial interesting amounts. This section studies ten gum exudates from genera within this family, which is widespread in subtropical North and South America. These gums have not been investigated previously although all have traditional and other uses within the local environments where the trees occur abundantly. They may therefore yet become articles of international trade.

9.1.1 Origin of Gum Samples

Cassia grandis L.f. and *Enterolobium cyclocarpum* (Jacq.) Griseb. gums were collected in Costa Rica by Professor D.H. Janzen, University of Philadelphia. The gum exudates from *Cercidium praecox* (Ruiz. and Pav.) Harms from Puebla, Mexico; *Caesalpinia sp. nov.* from Oaxaca, Mexico; *Lysiloma acapulcense* (Kunth) Benth.; *Senna nicaraguensis* (Benth.) Irwin and Barneby from Dept. Santa Rosa, Guatemala; and *Caesalpinia eriostachys* Benth. from Dept. Santa Ana, El Salvador were collected by G.P. Lewis and C.E. Hughes. *Parkia nitida* Miquel gum was sent for analysis by Avilio A. Franco, Rio de Janeiro. Gum exudates from *Prosopis flexuosa* D.C. and *Prosopis chilensis* (Molina) Stunts emend. Burkart are of Chilean origin.

9.1.2 Results and Discussion

Table 9.1(a) Analytical data for ten gum exudates (Leguminosae)

	Gum samples									
	Cassia grandis	*Senna nicar.*	*Cercid. praec.*	*Lysil. acapu.*	*Caesal. sp.nov.*	*Caesal. erios.*	*Enter. cyclo.*	*Parkia nitida*	*Proso. flex.*	*Proso. chil.*
H_2O%	16.1	17.5	12.7	14.1	14.1	18.1	11.1	13.3	12.4	11.5
Ash%	15.0	3.3	4.8	3.2	4.6	3.0	6.9	2.1	1.9	2.4
N%	0.63	0.44	1.8	0.30	0.66	0.25	0.26	0.37	0.58	0.57
NCF	5.15	6.33	5.93	6.47	5.87	6.07	6.31	6.83	6.56	6.73
Protein%	3.2	2.8	10.6	1.9	3.8	1.5	1.6	2.5	3.8	3.8
Methoxyl%	2.4	1.2	1.2	0.6	0.8	1.8	1.0	0.15	0.17	0.73
$[\alpha]_D$	+82°	+5°	-11°	-2°	+10°	-15°	+40°	-35°	+27°	+77°
Tannin%	0.3	0.27	0.22	0.9	0.15	0.25	0.25	0.36	0.8	3.4
[η]ml/g	10	216	34	5	190	16	67	7	9	6
E.Wt[a]	400	814	524	1240	625	1114	950	1240	1590	1070
U.A.A.	44	22	34	14	28	16	19	14	11	16
Sugar composition after hydrolysis%										
4-O-MGUA[b]	0	7	10	3.5	0	11	6	1	1	4
GUA	0	15	24	10.5	0	5	13	13	10	12
GAA	44	0	0	0	28	0	0	0	0	0
Gal	30	43	5	69	35	45	37	43	40	44
Ara	10	35	29	13	29	39	32	39	47	38
Xyl	16	0	32	0	8	0	0	0	0	0
Rha	tr	tr	tr	4	tr	tr	12	4	2	2

a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).
tr: trace

Table 9.1(b) Amino acid composition (per 1000 residues) for the Gum Exudates (Leguminosae)

	Gum samples									
	Cassia grandis	*Senna nicar.*	*Cercid. praec.*	*Lysil. acapu.*	*Caesal. sp.nov.*	*Caesal. erios.*	*Enter. cyclo.*	*Parkia nitida*	*Proso. flex.*	*Proso. chil.*
Ala	32	120	129	74	108	123	70	89	43	47
Arg	8	0	0	0	16	0	0	0	25	20
Asp	109	80	108	78	111	100	128	112	96	108
Cys	0	0	0	0	0	0	0	0	2	5
Glu	75	59	61	55	81	78	59	61	41	53
Gly	464	81	56	38	119	100	82	56	50	56
His	37	31	46	62	47	69	48	32	46	33
Hyp	68	28	80	207	41	37	86	174	198	192
Ile	7	34	40	0	42	34	34	36	29	27
Leu	13	56	77	37	55	60	54	56	62	54
Lys	37	72	56	34	104	60	86	27	27	29
Met	1	31	0	0	0	0	0	0	20	16
Phe	13	24	45	27	22	27	12	21	36	38
Pro	4	201	46	99	65	86	121	78	34	39
Ser	68	84	70	152	47	67	75	119	118	128
Thr	18	46	71	44	42	54	49	53	63	62
Tyr	29	25	23	16	11	21	31	31	47	47
Val	17	59	61	77	89	84	65	55	63	46
NCF	5.15	6.33	5.93	6.47	5.87	6.07	6.31	6.83	6.56	6.73

The analytical data for the physico-chemical, carbohydrate parameters and the amino acid compositions are shown in Tables 9.1(a) and 9.1(b); and for the cationic composition of the ash in Table 9.2.

Four of the gums studied (*Cassia grandis, Enterolobium cyclocarpum, Senna nicaraguensis* and *Caesalpinia eriostachys* were only ca. 50% soluble in cold water; the other samples had excellent solubility.

Neotropical *Cassia* was divided into three genera, viz. *Cassia sensu stricto, Senna* and *Chamaecrista* in 1982 (Irwin and Barneby 1982). As a result, only about 16 *Cassia (sensu stricto) spp.* are recognized. This is supported by the fact that the gums from *Cassia grandis* and *Senna nicaraguensis* differ significantly (Tables 9.1(a) and (b)). Thus, *Senna nicaraguensis* gum has a very high viscosity, a high Pro content and contains glucuronic acid, galactose and arabinose; *Cassia grandis* gum is of low viscosity, contains galacturonic acid, galactose, arabinose and xylose, and contains, in addition, a very large proportion of Gly and contents of manganese and zinc (Table 9.2) which must be regarded as high for a gum exudate.

Enterolobium is a small genus (ca. 8 species); members are native to tropical America and the West Indies (Allen and Allen 1981). *E. cyclocarpum* is one of the best known species, a fast-growing, large tree known as "elephant's ear" in Costa Rica. Its jelly-like exudate has been reported to be used in folk medicinal treatments (Allen and Allen 1981). Its gum has a composition and properties similar to those of the dextrorotatory, tannin-containing *Acacia* gums of the gum talha type (Anderson et al. 1966a) but its amino acid composition shows that this gum contains little Hyp and no Arg but high Asp and Pro. Its poor solubility decreases the number of local technological applications in which it can be used to replace imported gums. This gum contains exceptionally low and high proportions of calcium and potassium respectively.

Parkia nitida is abundant in the Amazon region and has been found to be an excellent local replacement for gum arabic in applications involving the pelleting and inoculation of legume seed with *Rhizobium* to assist rapid nodulation. In comparison with previous *Parkia* spp. studies (Anderson and Pinto 1985), *P. nitida* gum is very similar to *P. pendula* gum: both are laevorotatory and have very similar nitrogen contents, but the intrinsic viscosity of *P. pendula* gum (34ml/g) is considerably greater than that of *P. nitida* gum (7 ml/g). In addition, *P. nitida* gum contains a comparatively high amount of magnesium (Table 9.2).

Lysiloma, with ca. 35 species, is a Mexican genus with extensions into Central America and the West Indies. Extractions of the bark of various species are used for tannin (Allen and Allen 1981). Table 9.1 shows that *Lysiloma acapulcense* gum has a high tannin content. It also has a very low intrinsic viscosity, but its other analytical parameters are similar to those for gums from other Leguminous genera except that Ile is absent and comparatively high contents of Ser are present.

Caesalpinia is a large genus of ancient origin. Some gum exudates from this genus have been used in adhesives, in textile and in other native applications. Of the two *Caesalpinia* species, *Caesalpinia eriostachys* gum has a sugar composition that is devoid of rhamnose. It therefore has similarities to that of *Senna nicaraguensis* gum and is distinctly different from *Caesalpinia sp. nov.* gum, which is very viscous, contains galacturonic acid and xylose, and therefore can be regarded as having a closer affinity with the gum from *Cassia grandis* and *Cercidium praecox*. These findings are of interest to plant taxonomists.

Cercidium is a small genus, widespread in tropical and sub-tropical America, and

well-adapted to arid and semi-arid areas (Allen and Allen 1981). *Cercidium* was formerly congeneric with *Parkinsonia*. The gum from *Cercidium praecox* has high methoxyl and nitrogen contents, fairly high viscosity and a high Met content. Its most interesting features, however, are very high contents of xylose, galacturonic acid and very low content of galactose (<5%).

The genus *Prosopis* is a comparatively small, but complex leguminous genus, whose species delineation is confused by synonymy and variable forms. It is widespread in subtropical North and South America, Asia and Africa, but uncommon in Europe and Australia (Allen and Allen 1981). The gum exudates from *Prosopis* species (well-known as "mesquite gum") has been studied extensively (Cuneen and Smith 1948; Smith and Montgomery 1959; Dutton and Unrau 1963; Aspinall 1969; Aspinall and Whitehead 1970; Anderson and Farquhar 1982; Anderson et al. 1985b; Anderson 1989). *Prosopis* gums have mainly been used by pharmaceutical and chemical companies as a source of arabinose and galactose after hydrolysis. Nevertheless, *Prosopis* gum was proposed (Vernon Carter and Sherman 1980) as a possible cheaper alternative for gum arabic in food products, although it has been pointed out that the gums from *Prosopis* species have never been approved as food additives in the USA or in the EEC (Anderson and Wang 1989).

Prosopis chilensis is native to the Pacific Coast and arid region of Peru, Central Chile, Bolivia and Eastern and North-Western Argentina; *Prosopis flexuosa* is native to Northern Chile and Argentina. The gums from *Prosopis chilensis* and *Prosopis flexuosa* were dark brown in colour and gave strongly coloured reddish-brown or chocolate brown solutions. *P. chilensis* gum has a very high tannin content (3.4%) (Table 9.1). The analytical data show in Table 9.1 that the gum from *P. chilensis* gum has similarities to those from *P. alba, P. glandulosa, P. velutina* and *P. laevigata* studied previously (Anderson and Farquhar 1982) which form a "*P. glandulosa*" complex. Their gums do not differ greatly in terms of physico-chemical characteristics, with specific rotations ranging from +61° to +88°, nitrogen contents ranging from 0.35 to 0.95%, low intrinsic viscosity (5.5-12.6 ml/g), low proportions of rhamnose (1-5%) and ratios of Gal:Ara ranging from 32:48 (*P. alba*) to 44:40 (*P. laevigata*). In contrast, *P. flexuosa* gum differs appreciably from those of the *P. glandulosa* complex in having a much lower positive specific rotation (+27°) and lower uronic acid (11%) and methoxyl (0.17%) contents, although other parameters (e.g nitrogen content and amino acid composition) for these species are similar (Anderson et al. 1985b). The major difference between the *Prosopis* gums remains, however, that ascribed previously to *P. juliflora* gum (major source of commercial

mesquite gum), which alone has a negative specific rotation (-36°), together with a much higher rhamnose (13%) and lower nitrogen (0.19%) content, and higher ratio of Gal:Ara (45:24) (Anderson and Farquhar 1982). In addition, *P. chilensis* and *P. flexuosa* gums both have very high sodium contents (Table 9.2); the former is known to be a salt-tolerant species which thrives in saline soils. The chemical analytical parameters of *Prosopis* exudates may yield useful chemotaxonomic information regarding the fine points of botanical difference that arise in this taxonomically difficult genus.

Table 9.2 The cationic composition (μg/g ash) of the ash from ten gum exudates

	Gum samples									
cations	*Cassia grandis*	*Senna nicar.*	*Cercid. praec.*	*Lysil. acapu.*	*Caesal. sp.nov.*	*Caesal. erios.*	*Enter. cyclo.*	*Parkia nitida*	*Proso. flex.*	*Proso. chil.*
Ca	244000	43700	314000	221000	236000	121000	8800	35600	58500	174260
Cd	0	0	0	0	0	0	0	22	0	0
Co	<5	0	0	0	0	0	<5	40	12	3
Cr	<5	24	34	27	15	11	<5	16	62	27
Cu	19	0	0	0	0	0	21	81	234	104
Fe	350	277	137	852	87	76	225	705	216	972
Mg	41370	23500	60500	18500	14700	64760	10170	107000	41560	20110
Mn	1010	200	123	293	192	315	0	360	271	372
Na	2450	200	317	3460	2790	1980	3230	750	24015	19770
Ni	0	0	0	38	0	0	2	3	12	7
Pb	0	0	0	168	0	0	0	16	12	17
K	183700	261000	35400	201000	193000	126000	468750	121000	143470	135890
Zn	250	0	0	127	0	0	0	53	160	30

9.2 The Composition and Properties of Seven Gum Exudates

A wide range of tree exudates from other Families is used for a variety of technological uses in their countries of origin, but except for gum tragacanth (*Astragalus spp.*), gum karaya (*Sterculia spp.*) and gum arabic (*Acacia senegal* (L.) Willd.), gum exudates are prohibited for modern food and pharmaceutical use by international regulatory authorities.

9.2.1 Origin of Gum Samples and Results

Gums from the following origins were provided by the U.K. Tropical Products Institute: *Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae) and *Pseudocedrela kotschyi* Harms (Meliaceae), both from northern Nigeria; *Combretum paniculatum* Vent. subsp. *microphyllum* Klotsch. (Combretaceae) and *Cassine aethiopica* Thunb.

Table 9.3 Analytical data for some seven gum exudates

Items	Gum samples						
	Atalaya hemigla.	*Cassin. aethio.*	*Combr. panic.*	*Pseudo. kotsch.*	*Sclero. birrea*	*Sesbania sesban* var.nub.	*Sesbania sesban*
H_2O%	15.2	10.7	11.5	13.8	13.1	12.0	13.4
Ash%	2.3	0.5	3.5	4.1	7.1	n.d.	n.d.
N%	0.21	0.08	0.05	0.13	0.16	0.63	0.16
NCF	6.47	6.49	6.31	6.75	6.77	6.56	6.25
Protein%	1.3	0.5	0.3	0.9	1.1	4.1	1.1
Methoxyl%	0.9	0.05	2.4	0.54	0.75	n.d.	n.d.
$[\alpha]_D$	-33°	+25°	+11°	+23°	+42°	+96°	+109°
Tannin%	0.5	0.2	0.2	0.6	0.4	n.d.	n.d.
[η] ml/g	8	15	37	6	20	2	10
E.Wt[a]	810	1975	960	720	855	980	1320
U.A.A.	22	9	18	25	21	18	13
Sugar composition after hydrolysis%							
4-O-MGUA[b]	5.5	0.5	14	3	4.5	n.d.	n.d.
GUA	16.5	8.5	4	22	16.5	18	13
Gal	53	49	31	68	56	45	59
Ara	23	42	29	7	21	36	28
Rha	2	1	17	1	2	tr	tr
Man			5				
Amino acid composition (per 1000 residues)							
Ala	88	73	78	36	41	53	59
Arg	25	22	15	18	12	28	32
Asp	118	98	134	58	56	242	219
Cys	25	0	0	0	0	29	79
Glu	86	78	70	42	37	80	99
Gly	96	114	156	37	37	71	81
His	17	15	27	24	36	14	18
Hyp	98	118	109	353	348	0	13
Ile	35	34	33	17	17	22	47
Leu	48	72	52	50	56	27	49
Lys	42	46	44	48	25	25	38
Met	8	12	10	4	4	7	24
Phe	36	42	37	24	27	19	25
Pro	61	72	48	54	68	262	49
Ser	77	64	72	118	136	45	60
Thr	56	53	47	46	45	24	36
Tyr	26	22	23	25	15	24	36
Val	58	65	45	46	40	36	50
NCF	6.47	6.49	6.31	6.75	6.77	6.56	6.57

a.If all acidity arises from uronic acid.
b.If all methoxyl content located in this 4-O-Methylglucuronic acid(MGUA).

(Celestraciae) from Zimbabwe; *Atalaya hemiglauca* (S.Muell)S.Muell.ex Benth. from Alice Springs, Australia. A gum sample (ca. 2g) from *Sesbania sesban*(L.) Merr. var. *nubica* (Chiov.) was collected in Hawaii in 1988 and gum from *Sesbania sesban*(L.) Merr. was obtained from Kenya in 1989. In all cases only small amounts of gum were available and this restricted the extent of the studies that could be made (see Tables 9.3 and 9.4).

Table 9.4 The cationic composition (μg/g ash) of the ash from the seven gum exudates

	Gum samples						
Cations	*Atalaya hemigla.*	*Cassin. aethio.*	*Combr. panic.*	*Pseudo. kotsch.*	*Sclero. birrea*	*Sesbania sesban* var.nub.	*Sesbania sesban*
Ca	189000	182000	247000	248000	139000	n.d.	n.d.
Cd	3	0	1	8	11	n.d.	n.d.
Co	13	33	0	18	4	n.d.	n.d.
Cr	7	26	5	20	25	n.d.	n.d.
Cu	62	29	4	45	9	n.d.	n.d.
Fe	89	333	165	1390	1320	n.d.	n.d.
Mg	54800	56900	52400	31400	17000	n.d.	n.d.
Mn	107	600	547	125	40	n.d.	n.d.
Na	165	6940	150	270	980	n.d.	n.d.
Ni	2	37	3	8	1	n.d.	n.d.
Pb	4	11	11	21	76	n.d.	n.d.
K	67600	23800	40600	27100	18500	n.d.	n.d.
Zn	37	84	347	15	13	n.d.	n.d.

n.d.= not determined because of limited amounts of material.

9.2.2 Discussion

All of these gum samples dissolved readily in cold water. The solutions from *Sclerocarya birrea, Pseudocedrela kotschyi* and *Sesbania sesban* were dark brown in colour. The data in Table 9.3 show that the gum from *Atalaya hemiglauca* is of interest in having a negative specific rotation (-33°) very close to that of gum arabic from *Acacia senegal*; but it has a very low intrinsic viscosity (8 ml/g). Its amino acid composition also differs greatly from that of gum arabic, the key differences being the low Hyp and high Asp and Cys contents of *Atalaya hemiglauca* gum.

Cassine aethiopica is widespread in tropical Africa. The gum has low, but not quite exceptional, nitrogen, rhamnose and methoxyl contents and has a fairly low Hyp content (Table 9.3).

The *Combretum* gums are the most frequently occurring adulterants of commercial gum arabic. The sugar and amino acid composition of *Combretum paniculatum* gum are typical of the dextrorotatory *Combretum* gums (Anderson et al. 1986a; Anderson and Morrison 1990) having low nitrogen and high rhamnose contents and giving a more viscous solution than gum arabic. *Combretum paniculatum* gum has very high methoxyl and zinc contents (Table. 9.4). Its amino acid composition shows the features already established (Anderson and Morrison 1990) for *Combretum* gums, viz. high in Asp and Gly, relatively low in Hyp.

The gum from *Pseudocedrela kotschyi* was reported (Greenway 1941) to be used medicinally by natives in West Africa and as an arrow poison. It has very low arabinose and rhamnose contents and its amino acid composition has features (high Hyp and Ser) which are more commonly associated with the gums from *Acacia* and other genera within Leguminosae.

Sclerocarya birrea occurs widely in the drier African savannah regions, but also extends to Ethiopia and Uganda. Its sugar and amino acid compositions show similarities to those reported (Anderson et al. 1986b) for a sample of gum from *Sclerocarya caffra*, a species growing in Zimbabwe. The analytical parameters and amino acid composition of *Sclerocarya birrea* gum are similar to that from *Pseudocedrela kotschyi* gum and to those of *Sclerocarya caffra* gum from Zimbabwe (Anderson et al. 1986b).

The *Sesbania sesban* gums are strongly dextrorotatory with a trace of rhamnose and low viscosity. The major differences between the Hawaiian variety and the sample from Kenya are that *Sesbania sesban var. nubica* gum is considerably more proteinaceous, but much less viscous (2 ml/g) than the Kenyan sample. Their amino acid compositions also differ greatly although both have uncommon features in having relatively high Asp, Cys and Met contents, but an extremely low Hyp content. The *Sesbania sesban var. nubica* gum from Hawaii has much more Pro, but less Cys and Met than the Kenyan *Sesbania sesban.*

These seven gums are of poor quality. Because of their tannin contents, their use in foodstuffs in any country should be particularly avoided, but they may be useful for some technological purposes in local production areas.

Chapter 10
Structural Studies of Some Acacia Gums by Carbon-13 NMR Methods

^{13}C NMR spectroscopy has been applied in structural studies of gum exudates (Defaye and Wong 1986; Anderson and Wang 1990; Pinto 1991; Kapoor et al. 1991); the spectra provide a unique "fingerprint" of the gum in its natural form, which takes into account both its analytical and structural characteristics. From the finding that the chemical shifts of monosaccharides are similar to those of the corresponding constituent monosaccharide units within a polysaccharide, except for the substituent effects (Jennings and Smith 1982), the major sugar constituent units, linkages and configurations in the natural gum polysaccharides can be deduced from comparisons with the appropriate model sugars. The ^{13}C NMR spectrum profiles of different gum exudates therefore reveal the structural differences between them and can be used effectively to achieve the assignment of an "unknown" gum to a particular botanical origin. The major sugar units in *Acacia* genus gums are galactose and arabinose (taken together, ca. 70%), with smaller proportions of glucuronic acid and rhamnose (many *Acacia* gums only have a trace amount of rhamnose). The galactose, mainly in β-D-galactopyranose form, is normally located in the core of the highly branched structure, but arabinose has a range of forms; both α and β L-Arabinopyranose and L-Arabinofuranose are found in *Acacia* gums. The characteristics of the gums may well reflect the form(s) of arabinose present rather than the overall arabinose content.

In this Chapter, ten *Acacia* gum exudates (*A. arabica, A. karroo, A. laeta, A. mellifera, A. nubica, A. polyacantha, A. robusta, A. tortilis, A. goetzii, A. wanyu*) have been studied by ^{13}C NMR spectroscopy to reveal their main structural differences and to verify that the earlier structural studies of some of them by classical methods were correct.

10.1 Acacia arabica Gum

The gum exudate from *Acacia arabica* (Lam.) Willd. tree was reported to have a very high positive specific rotation (+112°) and to contain D-Gal (32%), L-Ara (57%), L-Rha (0.4%), D-GlupA (4%) and 4-O-Me-D-GlupA (6%) (Anderson et al. 1967).

Fig 10.1 is the ^{13}C NMR spectrum of *Acacia arabica* gum which shows great

Acacia arabica gum

ppm	Intensity
109.38	5.5
103.13	6.8
100.01	7.0
99.35	7.3
97.75	5.6
96.90	11.5
82.48	20.1
80.65	8.5
79.10	11.5
76.76	11.4
75.98	6.8
75.05	9.3
74.48	11.7
73.83	13.7
83.16	17.9
72.00	14.3
69.76	10.0
68.95	13.4
68.61	15.0
63.30	25.0
62.33	12.3
61.88	14.8
61.40	12.0
59.99	7.0

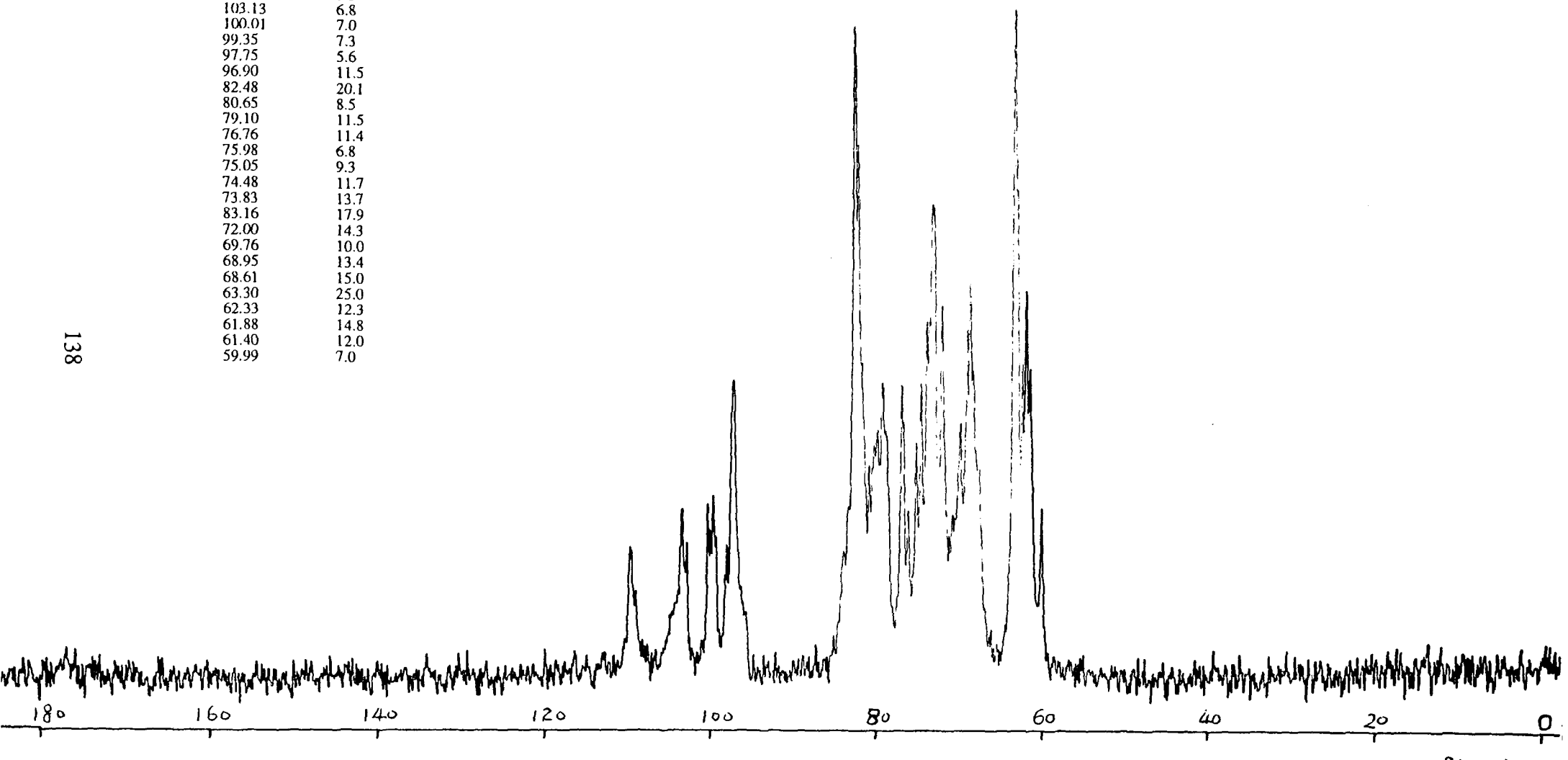

Fig. 10.1 ^{13}C NMR spectrum for *Acacia arabica* gum

structural differences when compared with that of gum arabic (*A. senegal*) (Fig. 4.1). There is an almost complete absence of rhamnose, more arabinose than galactose, and it also contains a significant methoxyl group content. Furthermore, more detailed information about the constituent sugar units and major linkages can be obtained (Table 10.1) from the spectrum; *A. arabica* gum mainly contains, in addition to D-Gal and β-L-Arap with (1→2) and 1,2,3 links, α-L-Araf which is mainly in terminal positions, and β-L-Araf with (1→2) links. There is no α-L-Arap. This confirms the previous methylation analysis studies which reported that there were Araf, Arap, (1→2) and (1→3)Araf, and (1→2)Arap in *A. arabica* gum (Anderson et al. 1967). The major peak at 82.5 ppm (Fig. 10.1) arises from C_3 in (1→3)β-D-Gal, C_4 in α-L-Araf, C_2 in (1→2)β-L-Araf and (1→3)β-L-Arap, the resonances being overlapped. The spectrum also shows the presence of the methoxyl group (60.0 ppm) present in 4-O-Me-β-D-GlupA (Gorin and Mazurek 1975).

Table 10.1 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.1) for *Acacia arabica* gum

Chemical shift (ppm)	C_1 of
109.4	α-L-Araf(1→ (with 61.4 ppm from C_5)
103.1	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.4 ppm from C_6)
(102.9)	→β-D-GlupA(1→ and β-D-GlupA(1→
100.0	→2)β-L-Araf(1→ (with 63.3 ppm form C_5)
99.4	β-L-Arap(1→ (with 63.3 ppm form C_5)
99.4	→)β-L-Arap(1→ (with 62.3 ppm from C_5)
97.7	→2)β-L-Arap(1→ (with 61.9 ppm from C_5)
96.9	→2,3)β-L-Arap(1→ (with 63.3 ppm from C_5)
60.0	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA

10.2 Acacia karroo Gum

Acacia karroo Hayne, belonging to Bentham's Gummiferae Series, is the most widespread *Acacia* in Southern Africa. It was reported that the composition of *A. karroo* gum from different African locations showed no great differences; the gum has a highly positive specific rotation (+38° to +67°), and consists of galactose

Acacia karroo gum

ppm	Intensity
109.39	9.4
107.52	6.8
104.45	5.5
103.24	8.6
100.74	4.8
99.90	12.0
97.63	9.9
84.79	4.7
84.05	7.8
82.80	10.6
82.03	16.5
81.84	18.3
80.72	7.9
79.73	9.5
79.12	8.9
76.56	13.4
76.18	14.8
75.17	6.9
73.95	12.6
73.28	15.2
72.85	16.7
72.01	14.4
69.87	15.2
68.75	23.4
67.07	12.3
65.73	3.9
63.20	15.9
62.45	8.5
62.05	8.0
61.40	25.0
60.10	7.0
20.55	3.1
16.55	4.3

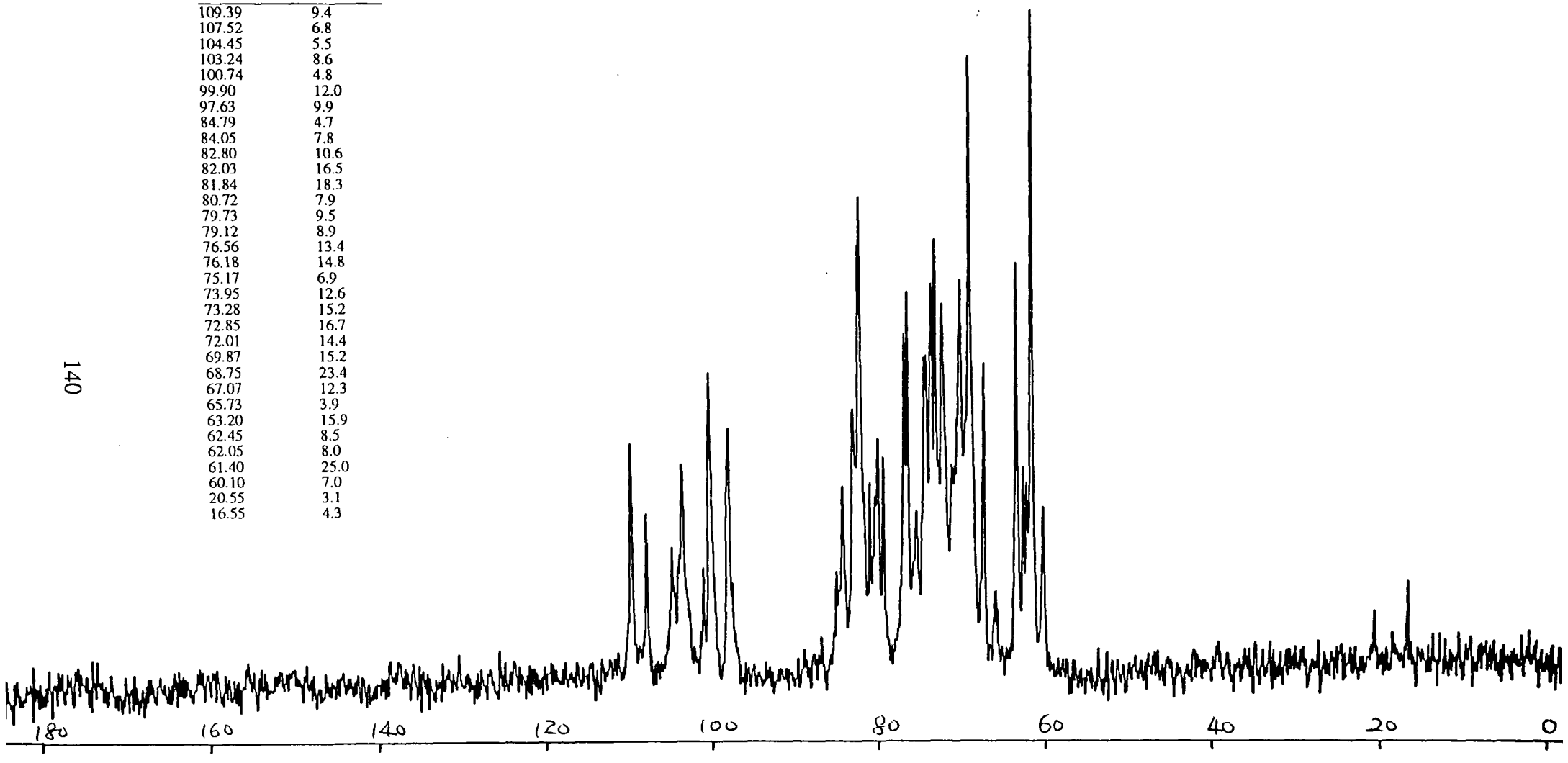

Fig. 10.2 ^{13}C NMR spectrum for *Acacia karroo* gum

(42-58%), arabinose (20-40%), rhamnose (4-10%) and glucuronic acid (14-17%) (Anderson and Pinto 1980).

Fig. 10.2 is the ^{13}C NMR spectrum for *A. karroo* gum from Zimbabwe. *A. karroo* gum contains both terminal and internal α-L-Araf (Table 10.2) in a ratio that is close to that in *A. senegal* gum (Fig. 4.1). Fig. 10.2 also shows that α-L-Arap (104.5 ppm), α-D-Gal (99.9 ppm) and internal β-L-Arap (97.6 ppm) are present in *A. karroo* gum, but that the rhamnose content is very low. Apart from the presence of 4-O-Me-β-D-Gal (60.0 ppm), a very small acetyl group content is also indicated (20.6 ppm) in *A. karroo* gum. This had not been detected by the chemical method in earlier studies.

Table 10.2 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.2) for *Acacia karroo* gum

Chemical shift (ppm)	C_1 of
109.4	α-L-Araf(1→
107.5	→α-L-Araf(1→Ara(1→ (with 61.4 ppm from C_5)
104.5	α-L-Arap(1→ (with 67.1 ppm from C_5)
103.3	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.4 ppm from C_6)
(102.9)	→β-D-GlupA(1→ and β-D-GlupA(1→
100.7	α-L-Rham(1→ (with 16.6 ppm from C_6)
99.9	→α-D-Gal(1→ and α-D-Gal(1→
99.9	→2)β-L-Araf(1→ (with 63.2 ppm from C_5)
97.6	→)β-L-Arap(1→ (with 62.5 and 62.0 ppm from C_5)
60.1	CH_3-O in 4-O-Me-β-D-GlupA
20.6	Carbon in acetyl group

10.3 Acacia laeta Gum

Acacia laeta R.Br. ex Benth., belonging to the Vulgares Series, is a natural hydrid of *A. senegal*(L.) Willd. and *A. mellifera*(Vahl.) Benth. and has two varieties viz. *A. laeta var. hashab* (which resembles *A. senegal*) and *A. laeta var. mellifera*, which resembles *A. mellifera*. It was reported that *A. laeta*'s gum has a negative specific rotation (-42°) and contained D-Gal (44%), L-Ara (29%), L-Rha (13%), D-GlupA (10.5%) and 4-O-Me-D-GlupA (3.5%) (Anderson 1978a). This is quite close to the

sugar composition of *A. senegal* gum. Previous methylation analysis studies (Anderson et al. 1968a) reported that terminal Araf and Arap, (1→3)Araf and (1→3)Arap were all present in *A. laeta* gum.

A ^{13}C NMR spectrum of the gum exudate from *A. laeta var. hashab* is shown in Fig. 10.3. Comparison with the spectrum of *A. senegal* gum (Fig. 4.1) shows that some similar features are present in the spectrum of *A. laeta* gum (Fig. 10.3). The arabinose present in both gums is mostly in the α-L-arabinofuranose form, but *A. laeta* gum contains more internal than external α-L-Araf, and less rhamnose than *A. senegal* gum. *A. laeta* gum (Table 10.3) also contains a small proportion of terminal and external α-L-Arap (C_5 at 66.4 ppm), (1→3)β-L-Araf (C_5 at 63.0 ppm), and (1→2)α-L-Araf (C_2 at 87.3 ppm); the latter was not reported in previous methylation analysis studies (Anderson et al. 1968a). The methoxyl group in 4-O-Me-β-D-GlupA is shown at 60.0 ppm (Fig 10.3).

Table 10.3 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.3) for *Acacia laeta* gum

Chemical shift (ppm)	C_1 of
109.5	α-L-Araf(1→
108.2	→α-L-Araf(1→
107.4	→α-L-Araf(1→Ara(1→ (with 61.2 ppm from C_5)
(104.5)	→α-L-Arap(1→ and α-L-Arap(1→ (with 66.4 ppm from C_5)
(103.9 -102.9)	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.2 ppm from C_6)
102.9	→β-D-GlupA(1→ and β-D-GlupA(1→
101.5	→3)β-L-Araf(1→ or β-L-Araf(1→ (with 63.0 ppm from C_5)
100.7	α-L-Rham(1→ (with 16.5 ppm from C_6)
60.0	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA

10.4 Acacia mellifera Gum

Analytical data for the gum exudate from *Acacia mellifera* (Vahl.) Benth., which belongs to the Vulgares Series, reported (Anderson and Farquhar 1978) that the gum had negative specific rotation (-56°), and contained D-Gal (43%), L-Ara (27%), L-Rha (9%), D-Glupa (11%) and 4-O-Me-D-GlupA (10%). This gum shows strong similarities with *A. senegal*, but its nitrogen content is five times higher than that of

Acacia laeta gum

ppm	Intensity
175.52	0.7
109.45	3.0
108.21	4.1
107.35	8.2
102.90	6.4
101.53	1.8
100.66	3.0
87.31	1.4
85.50	0.6
83.99	9.7
82.01	9.0
81.28	14.1
80.39	4.7
79.08	3.7
76.50	14.1
75.99	10.6
75.01	6.9
74.20	7.9
73.29	9.8
72.94	9.7
71.89	6.0
70.25	10.1
70.02	10.2
68.90	7.9
66.38	2.9
63.00	2.7
61.17	25.0
59.97	3.0
16.50	2.2

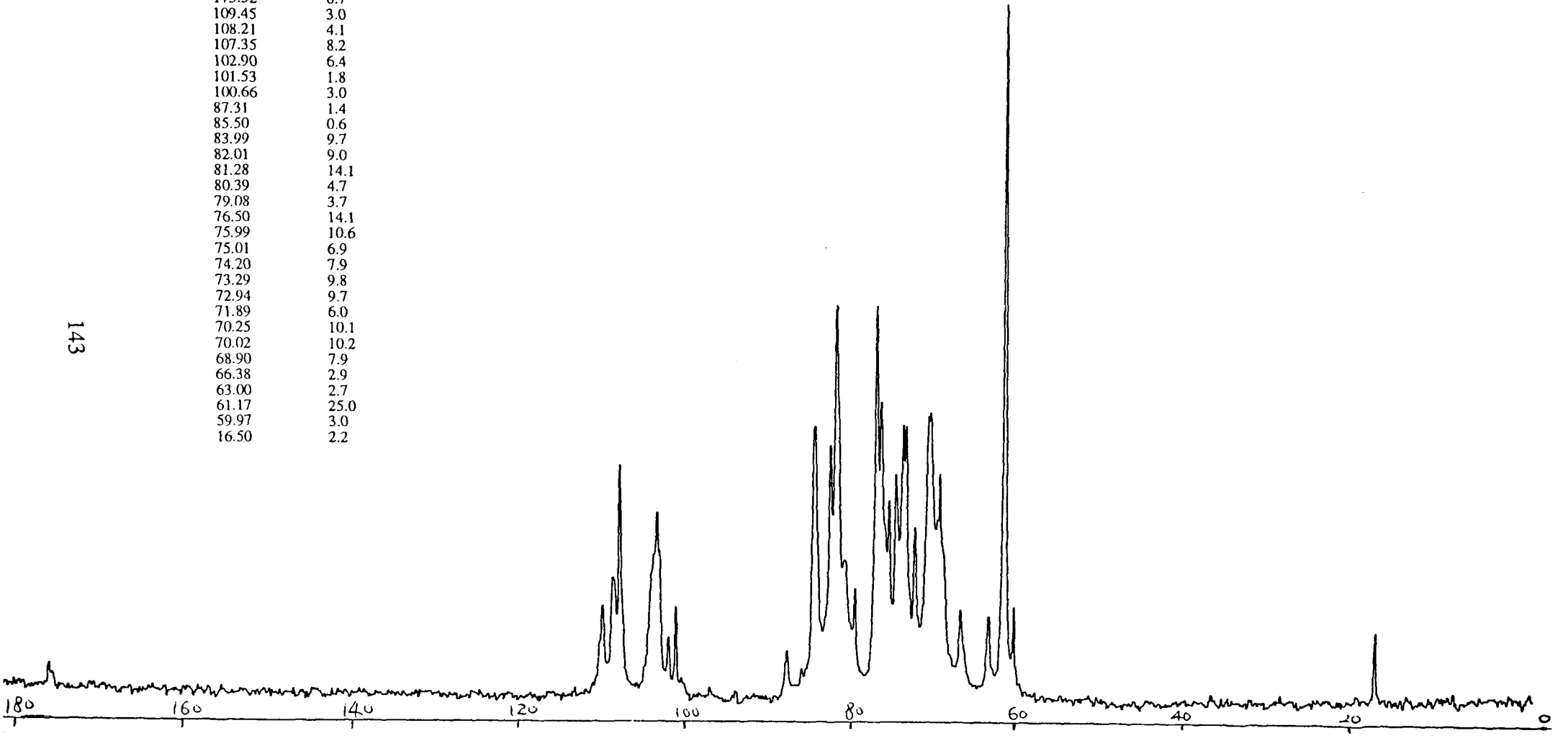

Fig. 10.3 ^{13}C NMR spectrum for *Acacia laeta* gum

A. senegal gum.

Table 10.4 lists the major sugar constituent units and linkages in *A. mellifera* gum shown by its ^{13}C NMR spectrum (Fig. 4.1).

A. senegal (Fig. 4.1), *A. mellifera* (Fig. 10.4) and *A. laeta* (Fig. 10.3) are very closely related botanically, but the spectra shows that structural differences do exist. α-L-Arabinofuranose (both external and internal) is the major form of arabinose in *A. mellifera* gum. Like *A. laeta* gum, *A. mellifera* gum also contains β-L-Araf (C_5 at 62.9 ppm) and a small amount of α-L-Araf (C_5 at 66.3 ppm). Although β-L-Araf is present the content is less than that in *A. laeta* gum. *A. mellifera* gum also contains a very small proportion of internal β-L-Arap (which is found neither in *A. senegal* nor in *A. laeta* gums) and a lower rhamnose content than *A. senegal* gum; 4-O-Me-β-D-GlupA is also present in the spectrum of this gum (at 60.0 ppm).

Table 10.4 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.4) for *Acacia mellifera* gum

Chemical shift (ppm)	C_1 of
109.8	α-L-Araf(1→
108.3	→α-L-Araf(1→
107.4	→α-L-Araf(1→Ara(1→ (with 61.1 ppm from C_5)
(<104.0)	→α-L-Arap(1→ (with 66.3 ppm from C_5)
(103.9 -102.9)	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.1 ppm from C_6)
102.9	→β-D-GlupA(1→ and β-D-GlupA(1→
101.5	→3)β-L-Araf(1→ or β-L-Araf(1→ (with 62.9 ppm from C_5)
100.7	α-L-Rham(1→ (with 16.5 ppm from C_6)
97.4	→2)β-L-Arap(1→ (with 61.6 -62.0 ppm from C_5)
96.7	→2,3)β-L-Arap(1→ (with 62.9 ppm from C_5)
60.0	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA

Acacia mellifera gum

ppm	Intensity
109.78	4.1
108.30	5.3
107.35	6.5
102.88	7.8
101.50	5.0
100.65	3.3
97.41	2.3
96.69	2.2
87.14	2.3
84.02	9.5
82.03	14.2
81.26	14.3
80.58	7.2
79.06	4.6
76.37	12.3
76.01	11.6
74.99	10.0
74.14	9.0
73.23	11.0
72.93	12.8
71.88	5.1
70.25	10.8
68.88	8.0
66.30	3.1
62.90	7.0
61.14	24.4
59.95	5.0
16.47	2.2

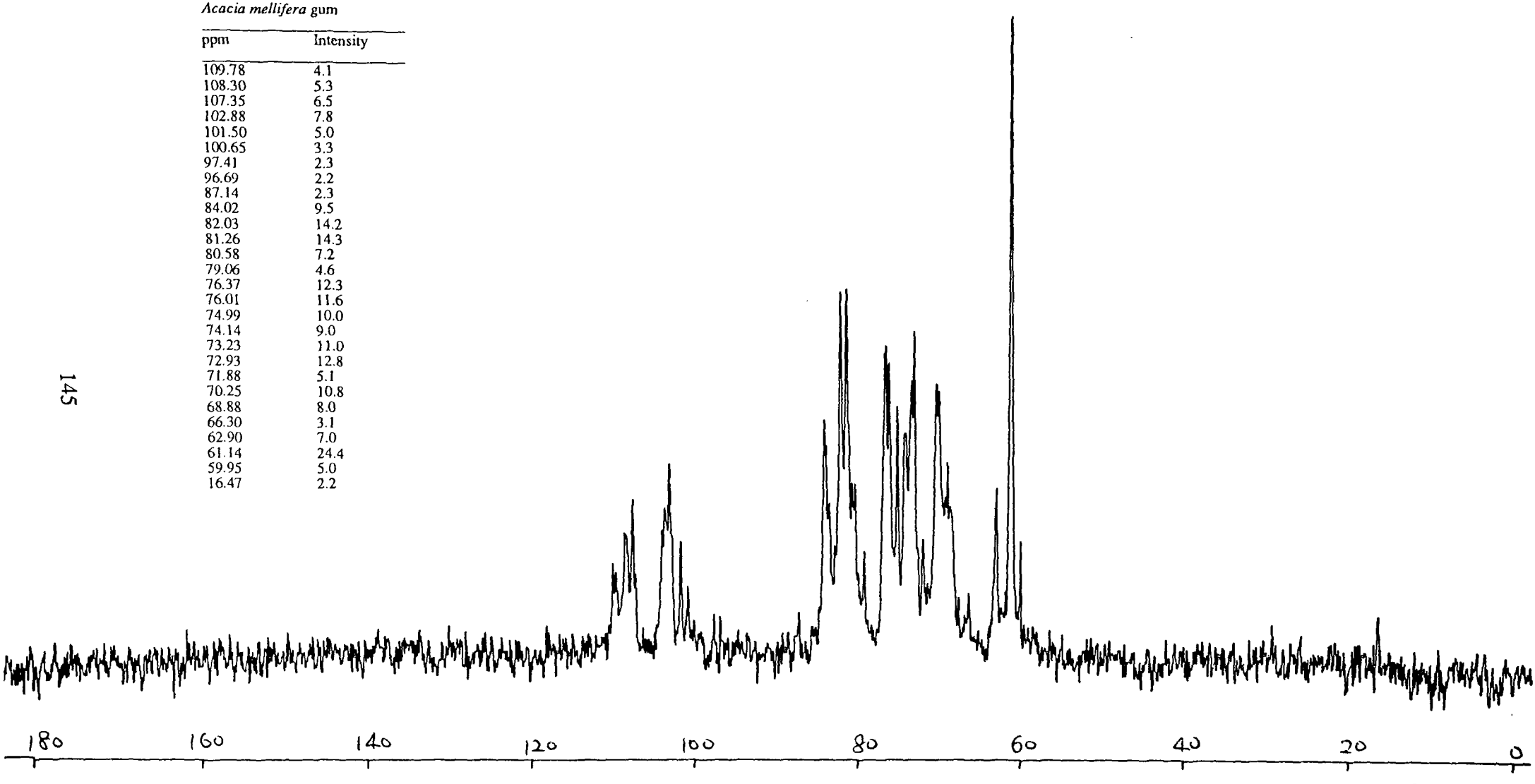

Fig. 10.4 ^{13}C NMR spectrum for *Acacia mellifera* gum

10.5 Acacia nubica Gum

The gum exudate from *A. nubica* Benth. (Gummiferae Series) was reported to differ in several interesting respects from *A. senegal*. *A. nubica* gum gave a highly positive specific rotation (+98°), very low methoxyl and rhamnose contents, but a very high arabinose content and a low nitrogen content (0.2%). It is very similar to *A. sieberana*. Previous studies (Anderson and Cree 1968a) showed that *A. nubica* gum contained D-Gal (33%), L-Ara (59%), L-Rha (1%), D-GlupA (6.5%) and 4-O-Me-D-GlupA (0.5%); terminal Araf and Arap, (1→2)Araf, (1→3)Araf and (1→2)Arap were the major arabinose forms and linkages identified by methylation studies (Anderson and Cree 1968a).

Fig. 10.5 shows the ^{13}C NMR spectrum of *A. nubica* gum. It is closely similar to that of *A. sieberana* gum (Fig.5.2) and this suggests structural similarities between *A. nubica* and *A. sieberana* gums. Table 10.5 lists the major sugar units and linkages in *A. nubica* gum indicated by its ^{13}C NMR spectrum. α-L-Araf, β-L-Arap and β-L-Araf are all present; furthermore, internal (1→2) and (1,2,3) linked β-L-Arap appear to be the major sugar units as deduced from the high intensities of the chemical shifts at 62.2 -63.3 ppm. α-L-Araf is also present in considerable amount. Methoxyl content (60.0 ppm) from 4-O-Me-β-D-GlupA is relatively low in this gum. Thus the structural features reported previously were correct.

Table 10.5 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.5) for *Acacia nubica* gum

Chemical shift (ppm)	C_1 of
(109.3)	α-L-Araf(1→
108.7	→α-L-Araf(1→ (with 61.4 ppm from C_5)
103.3	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.4 ppm from C_6)
102.9	→β-D-GlupA(1→ and β-D-GlupA(1→
99.5	→2)β-L-Araf(1→ (with 62.9 ppm from C_5)
99.0, 98.1	→)β-L-Arap(1→ (with 62.9, 62.3 ppm from C_5)
97.0	→2,3)β-L-Arap(1→ (with 63.3 ppm from C_5)
60.0, 60.1	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA

Acacia nubica gum

ppm	Integral	Intensity
108.67	5.4	3.4
103.27	1.9	1.8
102.94	1.8	2.1
99.53	1.5	2.1
98.07	5.7	3.9
82.52	32.0	19.3
81.76	9.1	8.2
81.51	1.5	5.8
81.33	4.5	6.7
81.14	7.2	5.9
79.73	6.9	4.8
79.17	12.7	10.1
78.65	28.2	14.1
76.79	10.2	8.0
74.59	10.4	8.8
73.61	11.4	8.8
73.13	24.4	14.4
72.16	3.8	4.7
71.92	2.4	4.4
71.64	4.7	4.8
69.93	3.7	3.4
69.78	3.0	3.0
63.30	29.7	20.9
62.92	9.4	10.1
62.31	11.9	7.4
61.40	20.2	9.9
60.18	1.1	1.7
60.02	1.4	1.6

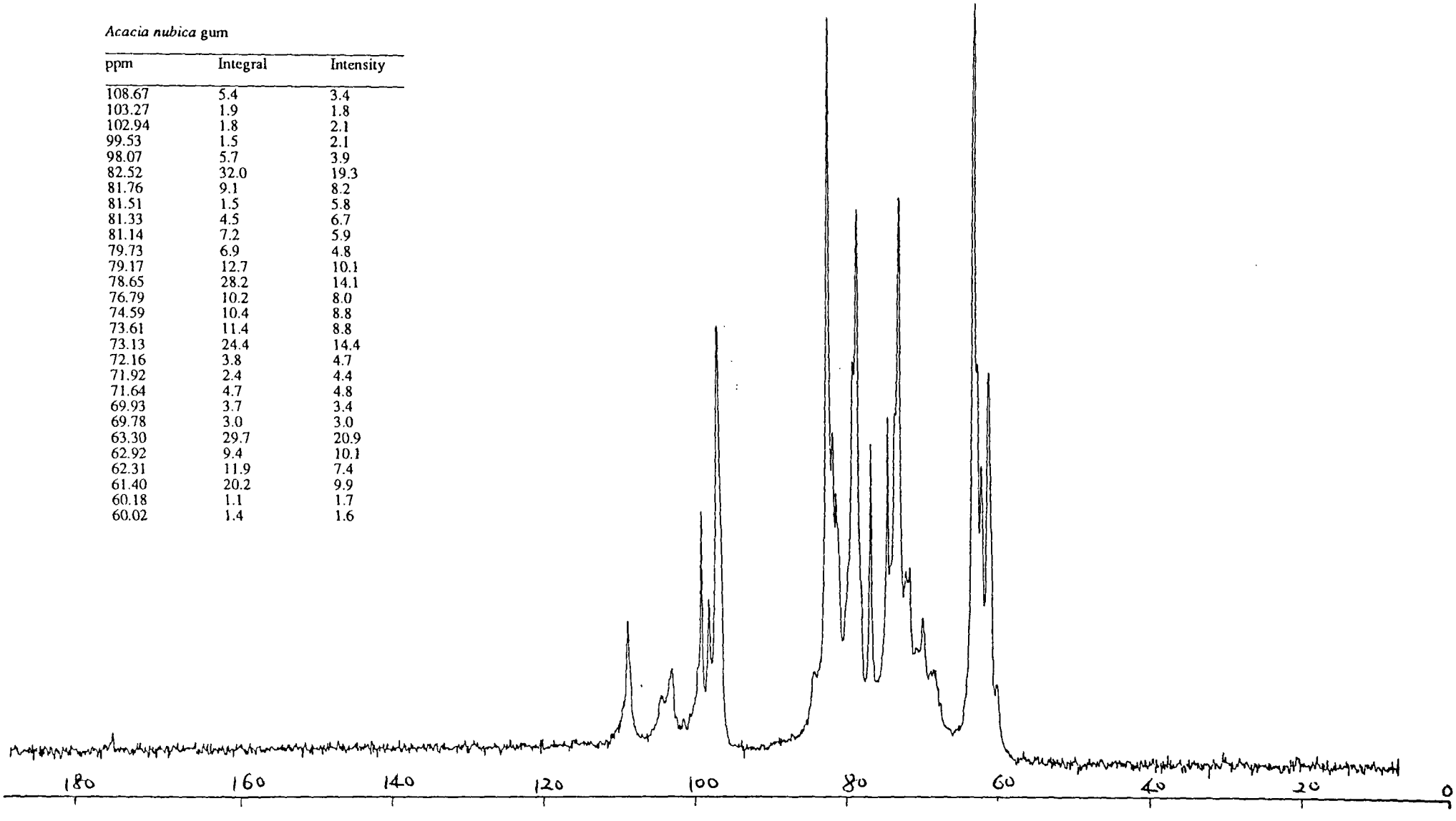

Fig. 10.5 ^{13}C NMR spectrum for *Acacia nubica* gum

10.6 Acacia polyacantha Gum

Acacia polyacantha Willd. subsp. *campylacantha* (Hochst. ex A. Rich.) Brenan (syn. *Acacia campylacantha*) is one of the species in Series 5 (Vulgares), sub-series 1 (Gerontogeae speciflorae) of Bentham's classification of the genus. Botanically, *A. polyacantha* is close to *A. senegal* (L.) Willd. and *A. laeta* R. Br. ex Benth. Previous studies showed that the gum from *A. polyacantha* contained D-Gal (54%), L-Ara (29%), L-Rha (8%), D-GlupA (7%) and 4-O-Me-D-GlupA (2%); both terminal Araf and Arap, (1→3) linked β-Arap and (1→3) linked β-Araf were the major arabinose forms and linkages found in *A. polyacantha* gum by linkage analysis and methanolysis (Anderson and Munro 1970).

Fig. 10.6 shows the ^{13}C NMR spectrum of *A. polyacantha* gum and the major sugar units and their linkages deduced are listed in Table 10.6. The constituent sugars and linkages indicated by the spectrum are greatly in agreement with the previous report (Anderson and Munro 1970). In comparison with the spectra of *A. senegal* (Fig. 4.1) and *A. laeta* (Fig. 10.3) gums, *A. polyacantha* gum (Fig. 10.6) shows differences in fine structure. *A. polyacantha* gum has a much smaller internal α-L-araf content (C_1 at 108-107 ppm) but a much larger β-L-Araf (C_1 at 101.4 ppm) and β-L-Arap (C_1 at 99.2 ppm) content than *A. senegal* and *A. laeta* gums. The rhamnose content in *A. polyacantha* gum is closer to that in *A. senegal* gum than *A. laeta* gum. The spectrum of *A. polyacantha* gum also shows that, like *A. senegal* gum, it has a quite low 4-O-Me-β-D-GlupA content.

Table 10.6 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.6) for *Acacia polyacantha* gum

Chemical shift (ppm)	C_1 of
109.4	α-L-Araf(1→ (with 61.2 ppm from C_5)
103.1	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.2 ppm from C_6)
102.6	→β-D-GlupA(1→ and β-D-GlupA(1→
101.4	→3)β-L-Araf(1→ or β-L-Araf(1→ (with 62.9 ppm from C_5)
100.7	α-L-Rham(1→ (with 16.5 ppm from C_6)
99.2	β-L-Arap(1→ and →β-L-Arap(1→ (with 62.9 ppm from C_5)

Acacia polyacantha gum

ppm	Intensity
109.36	9.8
103.14	7.2
102.61	5.8
101.39	8.5
100.70	7.5
99.18	5.8
83.81	7.1
83.58	6.7
82.64	7.6
81.95	9.8
81.29	3.9
80.04	7.8
79.78	8.9
79.05	8.5
76.25	10.5
76.07	8.1
75.08	3.1
74.12	13.6
73.30	13.0
71.92	8.7
71.29	9.2
70.24	11.6
70.00	15.4
69.78	13.8
69.22	25.0
68.90	15.2
68.43	11.8
68.19	11.7
62.85	9.6
61.24	21.9
16.50	7.6

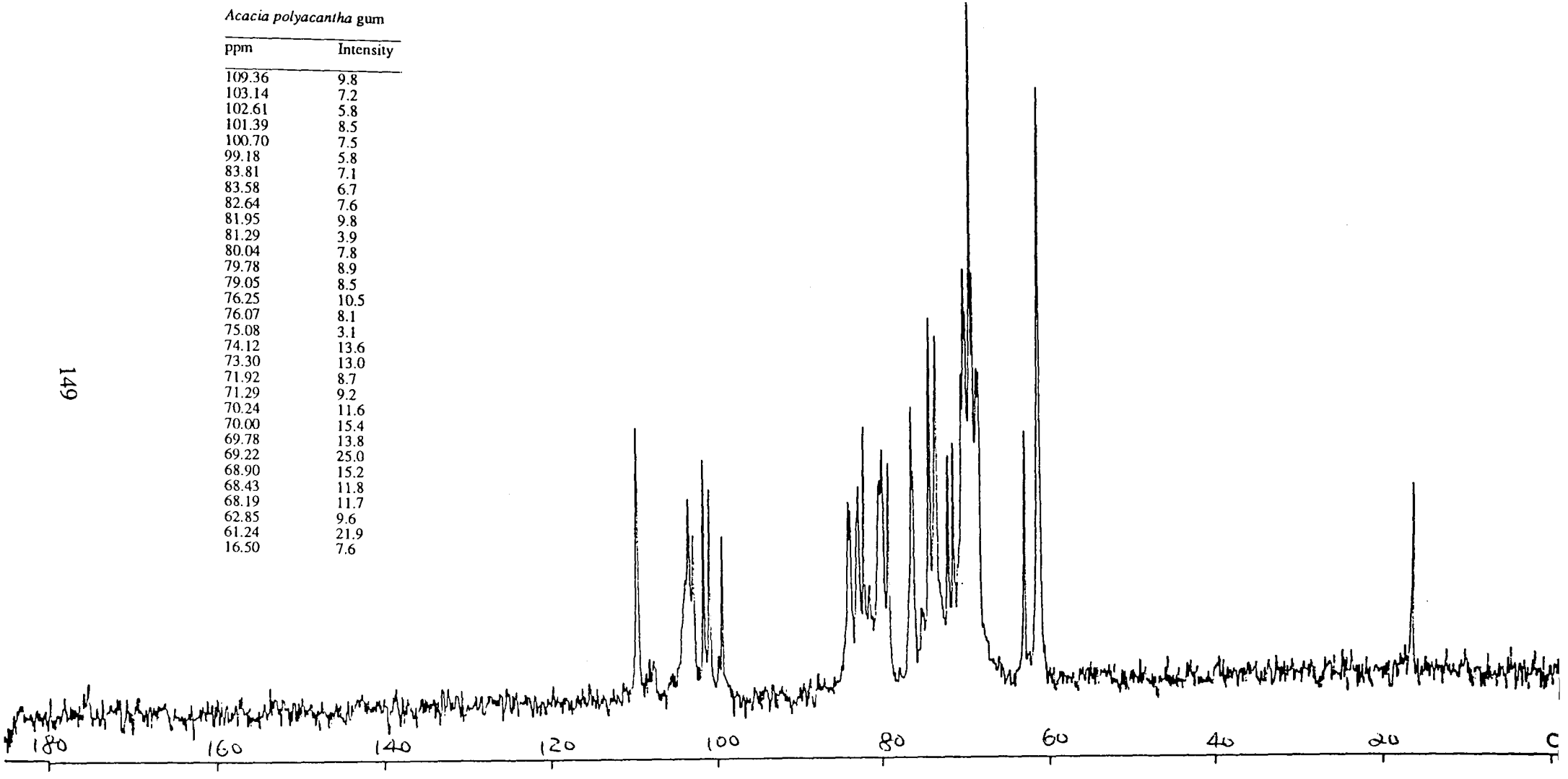

Fig. 10.6 ^{13}C NMR spectrum for *Acacia polycantha* gum

10.7 Acacia robusta Gum

The gum exudate from *Acacia robusta* Burch. ssp. *clavigera*, which is a member of Bentham's Series 4 (Gummiferae), sub-series 2 (Medibracteatae), was reported to give a positive specific rotation (+36°) and to have a fairly high protein content (18%) with the sugar ratios of Gal:Ara:Rha:Uronic acid=40:50:1:9 (Churms and Stephen 1984). Their structural studies showed a (1→3) linked galactan core with rhamnose at a peripheral position, also terminal Araf (11%) and Arap (6%), internal Araf with (1→2) links (6%) and (1→3) links (4%); and 11% of (1→2) linked or (1→4) linked Arap (Churms and Stephen 1984).

Fig. 10.7 shows the ^{13}C NMR spectrum of *Acacia robusta* gum. The sugar constituent units and linkages deduced from the spectrum are listed in Table 10.7 and are in agreement with the previous report. Furthermore, the spectrum shows that the terminal arabinose are mainly α-L-Araf and α-L-Arap, that the terminal Araf has mainly (→2)β-L-Araf and (→3)α-L-Araf linkages, and that the internal Arap is in the β-L-Arap form. The spectrum also shows a small acetyl content (20.4 ppm) and quite a high 4-O-Me-D-GlupA content.

Table 10.7 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.7) for *Acacia robusta* gum

Chemical shift (ppm)	C_1 of
109.3	α-L-Araf(1→
(107.2)	→α-L-Araf(1→Ara(1→ (with 61.3 ppm from C_5)
104.4	α-L-Arap(1→ (with 67.1 ppm from C_5)
103.1	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.3 ppm from C_6)
(102.9)	→β-D-GlupA(1→ and β-D-GlupA(1→
(100.5)	α-L-Rham(1→ (with 16.5 ppm from C_6)
99.6	→2)β-L-Araf(1→ (with 63.2 ppm from C_5)
97.6	→)β-L-Arap(1→ (with 62.3 ppm from C_5)
60.0	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA
20.4	carbon in acetyl group

Acacia robusta gum

ppm	Intensity
176.73	2.8
143.61	2.5
109.33	5.3
104.40	5.3
103.13	6.1
99.62	5.9
97.64	5.8
81.96	9.8
80.64	5.4
79.86	5.5
79.04	4.6
76.51	9.1
75.19	7.5
74.23	7.2
73.25	9.6
72.72	9.9
71.92	13.8
69.87	10.9
68.91	10.8
67.16	4.5
63.17	6.4
62.34	4.9
61.33	8.5
60.01	8.0
20.39	2.9
16.52	2.6

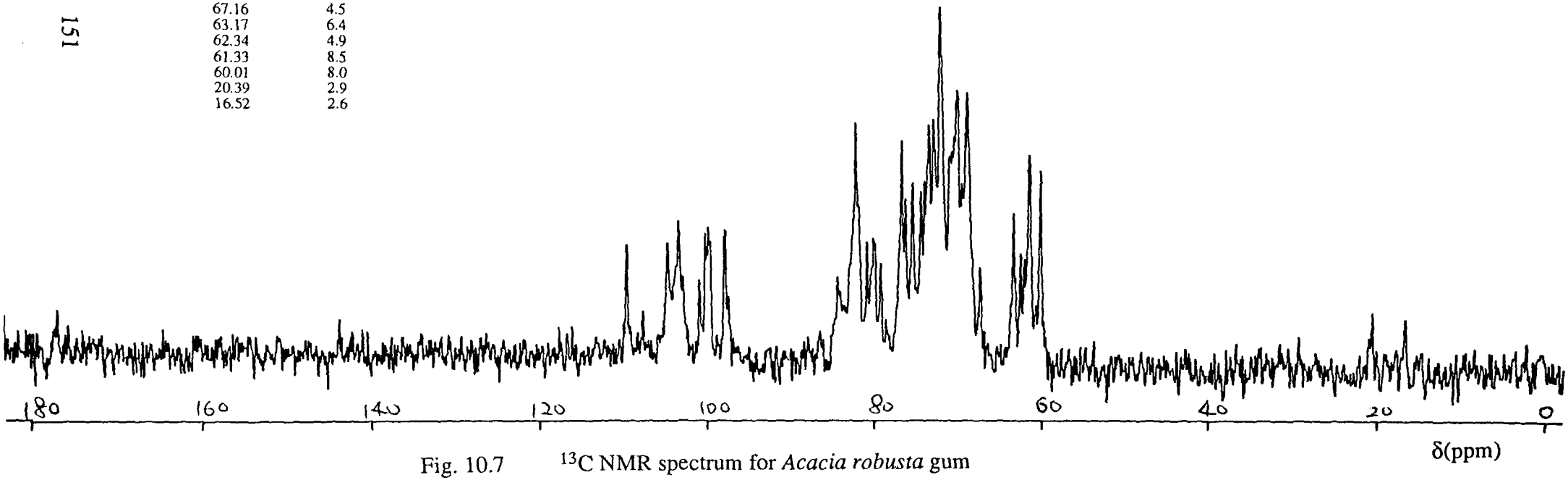

Fig. 10.7 ^{13}C NMR spectrum for *Acacia robusta* gum

10.8 Acacia tortilis Gum

Acacia tortilis (Forsk.) Hayne is a widespread species, complicated genetically, and apparently divisible into a number of distinguishable geographical races. There are four subspecies which can be distinguished: ssp. *tortilis*; ssp *spirocarpa* (Hochst. ex A. Rich.) Brenan; ssp. *heteracantha* (Burch.) Brenan; and ssp. *raddiana* (Savi) Brenan with two varieties viz. var. *raddiana* and var. *pubescens* A. Chev. (Anderson and Brenan 1975). A previous chemical analysis of gum samples from different *A. tortilis* subspecies showed many features in common; all gave positive specific rotations (from +74° to +97°), fairly high 4-O-Me-D-GlupA (3.5 -6.4%) and nitrogen (0.5 -1.5%) contents, more arabinose than galactose (the ratio ranging from 39:43 to 21:68), and a low rhamnose content (3-7%) (Anderson and Bell 1974). Methylation studies (Gammon et al. 1986) carried out on *A. tortilis* ssp. *heteracantha* gum showed that most of the arabinose presented was terminal Araf (12%), and Araf with (1→2) (38%) and (1→3) (11%) links.

Table 10.8 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.8) for *Acacia tortilis* gum

Chemical shift (ppm)	C_1 of
109.4	α-L-Araf(1→
(108.3)	→α-L-Araf(1→ (with 61.3 ppm from C_5)
104.5	α-L-Arap(1→ (with 67.0 ppm from C_5)
103.2	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.3 ppm from C_6)
(102.9)	→β-D-GlupA(1→ and β-D-GlupA(1→
100.7	α-L-Rham(1→ (with (16.5) ppm from C_6)
99.8	→2)β-L-Araf(1→ (with 63.1 ppm from C_5)
(99.7)	β-L-Arap(1→ (with 63.1 ppm from C_5)
97.6	→)β-L-Arap(1→ (with 63.1 ppm from C_5)
60.0	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA
20.5	carbon in acetyl group

Fig 10.8 is the ^{13}C NMR spectrum of *A. tortilis* gum from which the information can be deduced as listed in Table 10.8. It shows that the major arabinoses are terminal α-L-Araf with very small proportion of internal (1→3) linked α-L-Araf, and (1→2) linked β-L-Araf; this gum also contains significant proportions of α-L-Arap (C_1 at

Acacia tortilis gum

ppm	Intensity
177.44	1.9
109.37	8.0
107.53	2.2
104.48	4.6
103.12	7.9
100.70	3.0
99.83	13.6
97.59	11.3
84.02	6.9
82.65	6.7
81.77	15.5
79.68	8.7
69.06	4.6
67.48	6.8
76.08	14.6
75.26	5.8
74.10	12.6
73.37	9.9
72.68	17.0
71.88	12.9
70.85	12.0
70.20	13.7
69.80	14.1
68.70	21.3
66.99	10.8
63.13	12.4
61.28	25.2
60.00	7.3
20.50	3.0
16.54	2.3

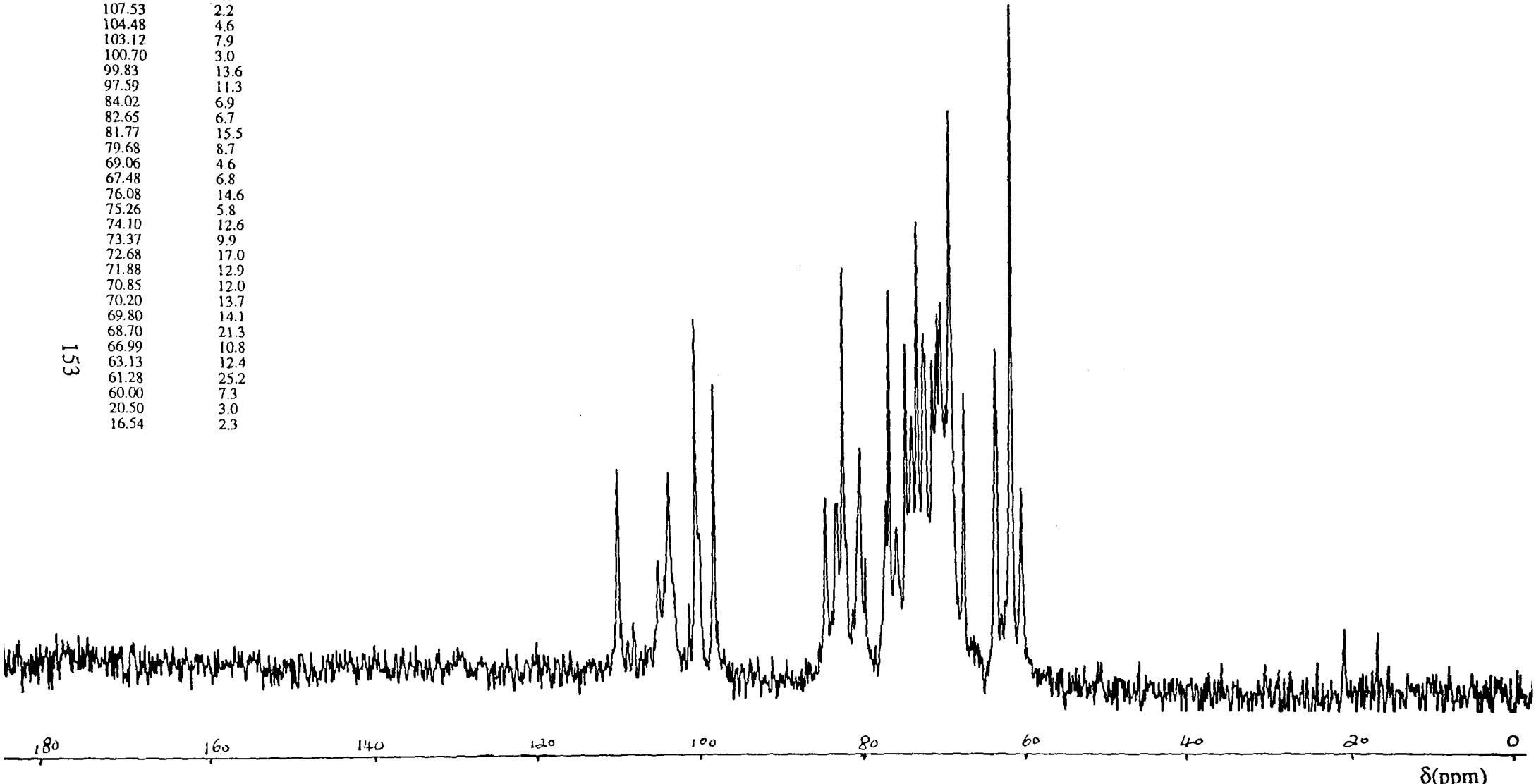

Fig. 10.8 ^{13}C NMR spectrum for *Acacia tortilis* gum

67.0 ppm) and β-L-Arap (C_1 at 63.1 ppm). Those arabinopyranose form sugars present in this gum were not reported by previous researchers (Gammon et al. 1986). The spectrum also shows a small acetyl content (20.5 ppm) and that methoxyl groups in 4-O-Me-D-GlupA (60.0 ppm) are present.

10.9 Acacia goetzii Gum

The gum exudate from *Acacia goetzii* subsp. *goetzii*, which belongs to the Vulgares Series, was reported to give a strongly negative specific rotation (-38°) and a sugar composition similar to that of *A. senegal* gum after hydrolysis: D-Gal (46%), L-Ara (22%), L-Rha (9%), D-GlupA (17%), and 4-O-Me-D-GlupA (6%) (Anderson and McDougal 1987b).

Fig 10.9 is the ^{13}C NMR spectrum for *A. goetzii* ssp. *goetzii* gum. The information deduced from the spectrum is listed in Table 10.9. In comparison with the spectrum of gum arabic (Fig. 4.1), significant differences in fine structure are observed. *A. goetzii* ssp. *goetzii* gum contains a large proportion of β-L-Araf (C_1 at 62.9 ppm) but the α-L-Araf content is much lower than in *A. senegal* gum. There is, however, much more (1→6) linked D-Gal in this *A. goetzii* gum than in *A. senegal* gum. In addition to 4-O-Me-β-D-GlupA, the presence of 4-O-Me-α-D-GlupA is also indicated (C at 57.4 ppm) in this spectrum. Also a split rhamnose CH_3 resonance (at 16.8 and 16.5 ppm) is observed, suggesting at least two different linkage environments for rhamnose.

Table 10.9 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.9) for *Acacia goetzii* ssp *goetzii* gum

Chemical shift (ppm)	C_1 of
109.4	α-L-Araf(1→
108.2	→α-L-Araf(1→ (with 61.3 ppm from C_5)
(<104.0)	→α-L-Arap(1→ (with 66.4 ppm from C_5)
103.7	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.3 ppm from C_6)
102.9	→β-D-GlupA(1→ and β-D-GlupA(1→
101.5	→3)β-L-Araf(1→ or β-L-Araf(1→ (with 62.9 ppm from C_5)
100.7	α-L-Rham(1→ (with 16.8 and 16.5 ppm from C_6)
60.0	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA
57.4	$\underline{C}H_3$-O in 4-O-Me-α-D-GlupA

Acacia goetzei ssp. goetzei gum

ppm	Intensity
109.39	2.1
108.18	2.2
103.68	6.3
102.92	8.5
101.50	5.4
100.65	13.3
83.55	4.2
81.98	10.3
81.30	6.3
79.03	12.4
76.10	15.6
74.19	19.0
73.28	20.1
71.95	16.1
70.26	19.6
70.01	25.0
68.90	21.3
66.34	1.8
62.94	7.8
61.31	9.4
59.95	2.3
57.42	1.8
16.80	5.0
16.52	13.6

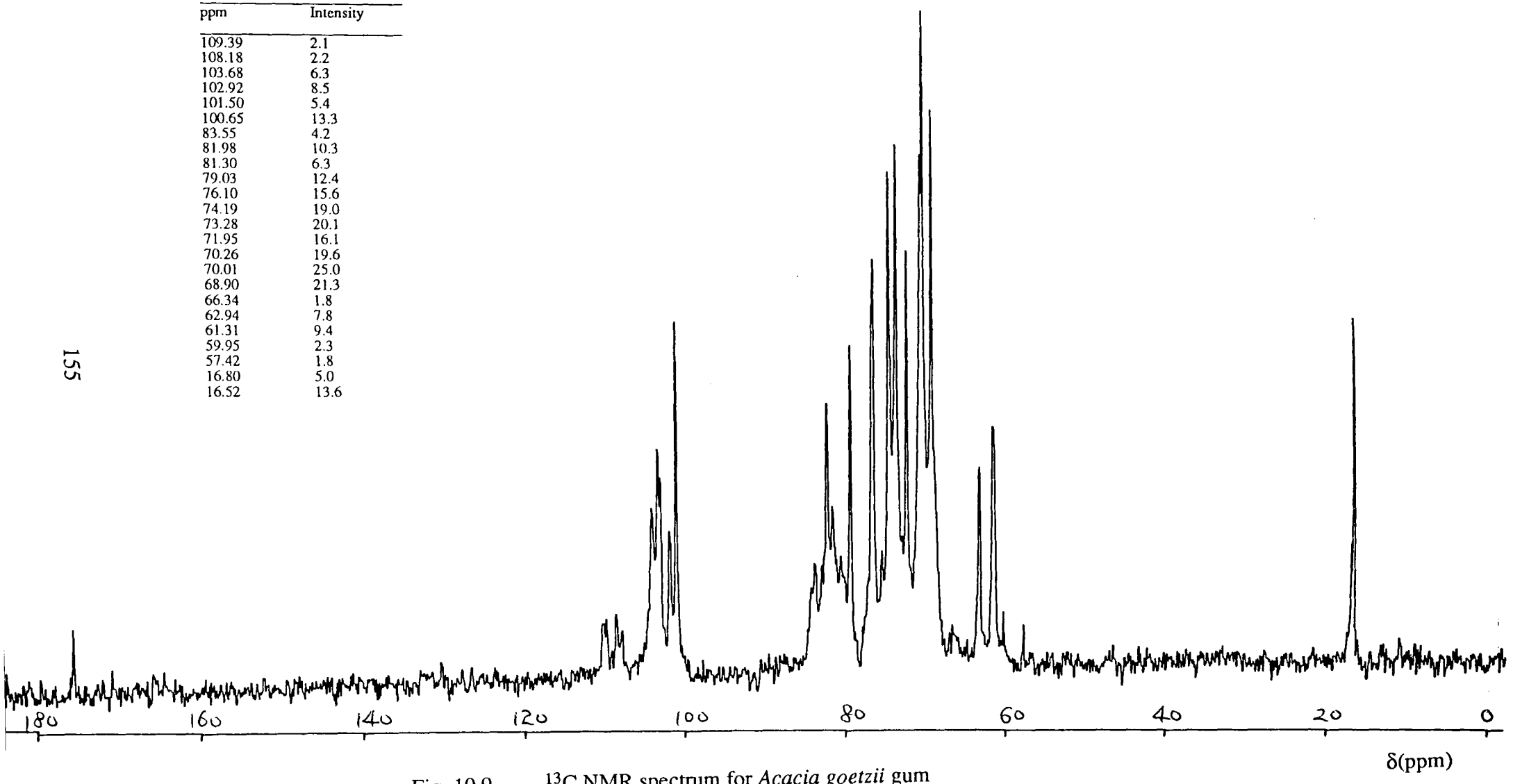

Fig. 10.9 ^{13}C NMR spectrum for *Acacia goetzii* gum

10.10 Acacia wanyu Gum

A ^{13}C NMR spectrum of *Acacia wanyu* gum, whose physico-chemical parameters have not been reported so far, is shown in Fig. 10.10. The sugar units present and their linkages are listed in Table 10.10. *A. wanyu* gum contains mainly galactose, (1→3) and (1→6) linked, and also has a very high methoxyl group content in the 4-O-Me-D-GlupA form (60.0 ppm); there is a considerable amount of rhamnose but very low arabinose contents. Of the arabinose residues in this gum, terminal β-L-Arap (C_1 at ca. 63 ppm) is the major form and with a very small amount of terminal α-L-Araf (C_1 at 109.4 ppm) and internal β-L-Arap (C_1 at ca. 63 ppm) also present.

Table 10.10 The composition of sugar units indicated by the anomeric peak assignments in the ^{13}C NMR spectrum (Fig. 10.10) for *Acacia wanyu* gum

Chemical shift (ppm)	C_1 of
109.2	α-L-Araf(1→ (with 61.0 ppm from C_5)
103.6	→β-D-Gal(1→ and β-D-Gal(1→ (with 61.0 ppm from C_6)
102.5	→β-D-GlupA(1→ and β-D-GlupA(1→
100.7	α-L-Rham(1→ (with 16.5 ppm from C_6)
99.6	β-L-Arap(1→ (with (63.2 -62.8) ppm from C_5)
97.7	→)β-L-Arap(1→ (with (61.7) ppm from C_5)
60.0	$\underline{C}H_3$-O in 4-O-Me-β-D-GlupA

Overall conclusions: This work has supplied a great opportunity to check the results obtained previously for nine of these gums by classical chemical analytical methods. The information obtained from their ^{13}C NMR spectra indicate that the structural data given in the previous reports were almost always correct. Because of the assignments now known it is also very effective to use ^{13}C NMR spectra to establish the structural features of "unknown" gums as shown by this study of *A. wanyu* gum.

Acacia wanyu gum

ppm	Intensity
175.71	2.3
109.19	2.5
103.57	14.4
102.52	10.7
100.66	6.0
99.55	3.7
97.71	2.1
82.02	14.1
79.00	6.6
75.99	14.8
75.04	23.1
74.17	12.2
72.72	23.8
72.01	19.8
70.69	25.0
70.21	21.2
68.61	22.8
61.02	21.0
59.96	15.0
16.49	5.5

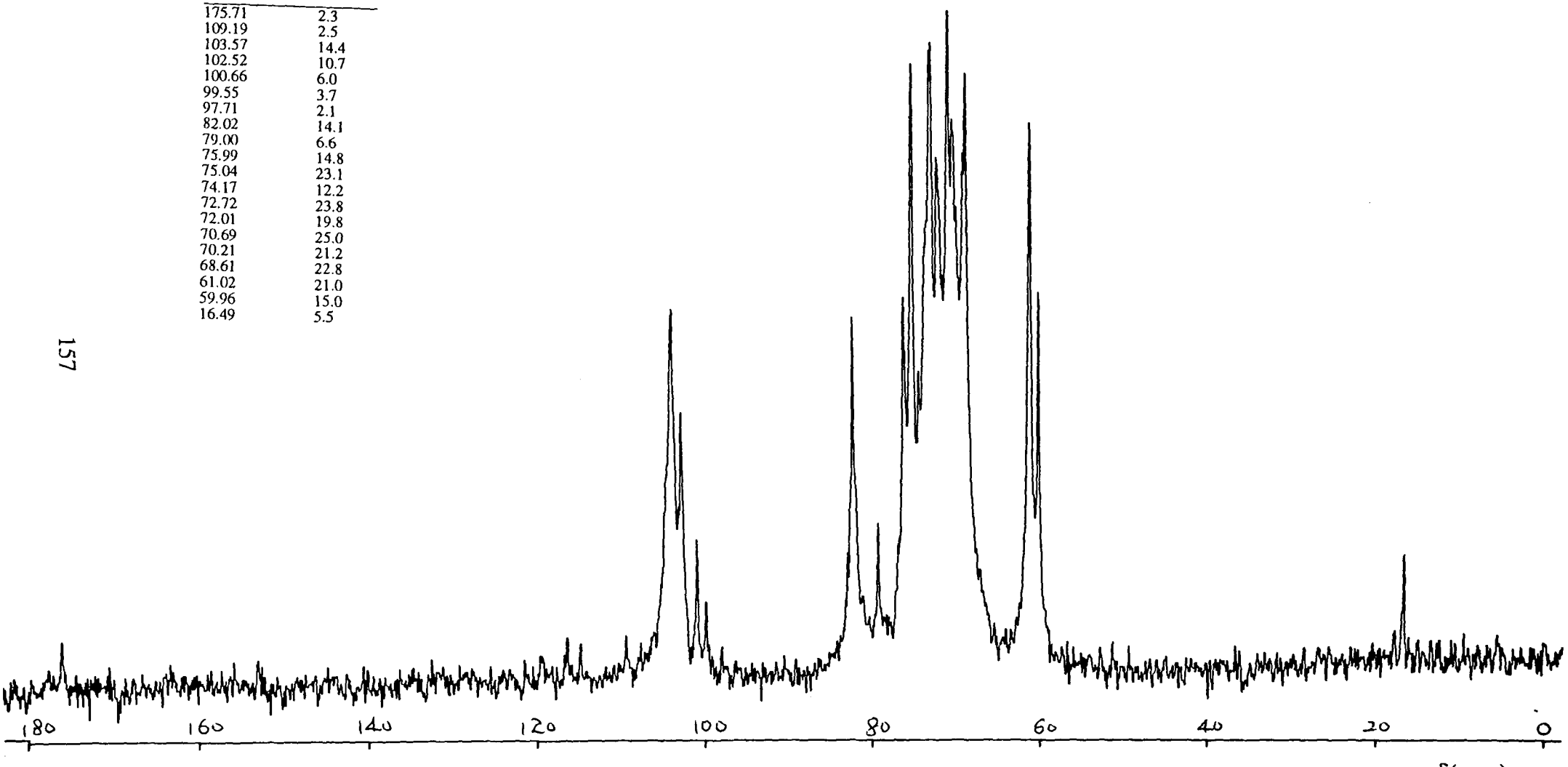

Fig. 10.10 ^{13}C NMR spectrum for *Acacia wanyu* gum

Final Conclusions

Forty five different gum exudates have been examined in this Thesis, including twenty one from the *Acacia* genus; six from the genus *Leucaena*; two each from the *Combretum, Prosopis, Sesbania* and *Caesalpinia* genera; and one each from the *Atalaya, Sclerocarya, Pseudocedrela, Senna, Cassia, Cassine, Cercidium, Parkia, Lysiloma and Enterolobium* genera. For the commercially important gums such as gum arabic (*A. senegal*), gum tahla (*A. seyal*) and Combretum gum, samples from different locations and the associated soils were also examined. In addition, more than thirty ^{13}C NMR spectra for different gums have been presented so that in future their "fingerprint" patterns are available as well as the previous chemical analytical data to provide a basis for the rapid identification of the true botanical origin of a gum whose origin is in dispute. This is now necessary for regulatory purposes as a result of the tendency for some gum suppliers to try to exploit loopholes in the existing international specifications.

The ^{13}C NMR spectra for the fractions and degraded products from some gums are also presented in order to reveal structural details. Furthermore, ^{13}C NMR spectra obtained for the mixtures of neutral sugars released from gums after acidic hydrolysis can be used to obtain quantitative results under defined operating conditions; the sugar ratios can be easily calculated from the intensities of the C_1 resonances as expressed by the relationship:

$$\text{Gal:Ara:Rha:...=Int Gal}_{\alpha+\beta}\text{:Int Arap}_{\alpha+\beta}\text{+Int Araf}_{\alpha+\beta}\text{:Int Rham}_{\alpha+\beta}\text{...}$$

Such spectra for gums studied 20 -30 years ago have shown that the relative proportions of the neutral sugars present, as determined earlier by chromatographic methods, were generally accurate. The NMR method has revealed that free arabinose, galactose and rhamnose are not degraded under the mild acidic conditions that were used in earlier studies but that the uronic acids were slightly susceptible to degradation, so tending to lead to earlier under-estimations of their actual content.

Since the information deduced from the ^{13}C NMR spectra for natural gum exduates has confirmed earlier quantitative analytical results, and supported the structural studies made by the earlier, much slower, classical methods, the spectroscopic correlations available are now very extensive and can be used to facilitate further studies that could be carried out in a more rapid way.

It is hoped that the work undertaken in this Thesis can be accepted as a contribution towards increasing the previous state of knowledge of the chemistry of plant gums, which have always been regarded as being the most complex of the natural polysaccharides, and are now commonly regarded as proteoglycans as a result of the reports of their peptide/protein content first made by workers in this laboratory over twenty years ago.

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Appendix A
Abbreviations

Ara	L-Arabinose
Araf/Af	L-Arabinofuranose
Arap/Ap	L-Arabinopyranose
ca.	circa
cp	centipoise
EA	Emulsifying Activity
ES	Emulsification Stability
E.Wt.	Equivalent Weight
GA	Gum arabic(*Acacia senegal*(L.)Willd.)
Galp	D-Galactopyranose
GalpA	D-Galacturonic acid
Glup	D-Glucopyranose
GlupA	D-Glucuronic acid
GUA	D-Glucuronic acid
Int	Relative intensity of NMR spectrum
Intg	Relative Integral of NMR spectrum
Manp/Man	D-Mannopyranose
MGUA	Methylglucuronic acid
Mw	Molecular weight (daltons)
N%	Nitrogen %
NCF	Nitrogen conversion factor
n.d.	Not determined
Rham	L-Rhamnose
Rec.	Recovery or yield
d	Smith-degradation dialysate product
SD	Smith-degradation product
U.A.	Uronic acid
U.A.A.	Uronic acid anhydride
w/v	weight/volume
w/w	weight/weight
Xyl	D-Xylopyranose
$[\alpha]_D$	Specific rotation at room temperature (degrees)
$[\eta]$	Intrinsic viscosity at 25±0.1°C (ml/g)
δ	Chemical shift (ppm) of ^{13}C NMR spectrum
AA	Amino acid
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
Cys	Cystine
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Hyp	Hydroxyproline
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine

Thr	Threonine
Tyr	Tyrosine
Val	Valine
Try	Tryptophan
Al	Aluminium
Ba	Barium
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
K	Potassium
Mg	Magnesium
Mn	Manganese
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
Si	Silicon
Sr	Strontium
Ti	Titanium
Zn	Zinc

Appendix B
List of Postgraduate Lectures Attended

1. "Short Course in X-Ray Fluorescence Spectrometry- Part One" by Dr. Fitton (Geology Dept. Edinurgh Univ.), 29th November 1989.

2. "Short Course in X-Ray Fluorescence Spectrometry- Part Two" by Dr. Fitton (Geology Dept. Edinurgh Univ.), 6th December 1989.

3. "Carbohydrate Beginnings to a Variety of Ends" by Dr. D.A.Rees, FRS (Medical Research Council), 13th March 1990.

4. "Recent Advances in Inorganic & Structural Chemistry" by Prof. Ebsworth et al., in T40, 14th March 1990.

5. "Development of Anti-cholesteremia Agents- Part 1 & 2" by Prof. R.Baker (Merck Sharp & Dohme, U.K.) in T100, 15th March 1990.

6. "Current Topics in Organic Chemistry" by Prof. Ramage et al., in T250, 16th March 1990.

7. "Overview and Introduction to Computer Techniques" by Dr. K.P.Lawley, in T100, 23rd April 1990.

8. "Computers in Chemistry: Fourier Transform & Spectrum Processing" by Dr. G.S.McDougall, 7th May 1990.

9. "Computers in Chemistry: Molecular Mechanics & Modelling" by Dr. P.Taylor, 28th May 1990.

10. "Biological Two-electron Oxidations: The Hydride and Carbanion Mechanisms" by Dr. S.K.Chapman, 29th May 1990.

11. "New Materials for the 21th century: The Role of Organic Chemistry" by Dr. G.Tennant, 5th June 1990.

12. "Aspects of Co-enzyme Chemistry and Biology" by Dr. R.L.Baxter, 5th June 1990.

13. "Computers in Chemistry" by Dr. M.H.Palmer, 11th June 1990.

14. "The Characterization of Metal-binding Sites in Proteins by NMR and Other Techniques" by Dr. S.K.Chapman, 18th June 1990.

15. "Protein Crystallography 25 Years after Lysozyme" by Sir D.Phillips, KBE, FRS in T250, 19th October 1990.

16. "Synthesis of Nerve Growth Factor Peptides" by Dr. D.Tumelty, in T100, 29th October 1990.

17. "Synchrotron Radiation and Chemistry", by Dr. G.N.Greaves (SERC Daresbury Lab.), 5th April 1991.

18. "Molecular Spectroscopy" by Dr. A.Hopkirk (SERC Daresbury Lab.), 5th April 1991.

19. "Surface Chemistry" by Dr. E.A.Seddon (SERC Daresbury Lab.), 5th April 1991.

20. "Electron Spectroscopy and Secondary Ion Mass Spectrometry for Surface analysis" by Dr. S.Affrossman (Chem. Dept., Strathclyde Univ.), 10th June 1991.

21. "Infrared Methods for Surface Analysis" by Dr. G.S.McDougall (Chem Dept., Edinburgh Univ.), 11th June 1991.

22. "Surface analysis using Laser Ionisation Mass spectrometry" by Dr. P.John (Chem. Dept., Heriot-Watt Univ.), 13th June 1991.

23. "Introduction to X-Ray Structure Determination" by Drs. R.O.Gould and A.J.Blake, 28th June 1991.

24. "Eurocarb VI European Symposium on Carbohydrate Chemistry", Heriot-Watt University, 8-13 September 1991.

25. "Detergent Products Around the World, etc." by Dr. C.J.Adams, Unilever Research,Port Sunlight Laboratory, 10th December 1991.

26. "Structure Property Relationships, & Applications of Aromatic Polymers, etc." by Dr. David Parker, ICI Wilton, 11th December 1991.

27. "ICI Pharmaceuticals Presentation on Discovery, Development & Pharmacology of Zoladex for Treatment of Prostate Cancer" by Drs. B.J.A.Furr, A.S.Dutta, et al., in T100, 27 March 1992. (6 lectures)

28. "Aspects and Applications of NMR spectroscopy 1992" by Dr. I.H.Sadler, in T100, 4-5 June 1992. (5 lectures)

29. "Recent Advances in the Synthesis and Activity of Agrochemicals" by Dr. I.Boddy and Dr. P.J.Dudfield (Schering Agrochemicals), in T100, 9-10 June 1992. (6 lectures)

30. "Mass Spectrometry in Action" by Professors J. Monaghan (ICI, Runcorn) and J. Scrivens (ICI Wilton), in T100, 7-8 January 1993. (6 lectures)

31. Attendance at annual one-week computer courses, 1989 to 1992.

Appendix C
Publications

1. **Food Hydrocolloids**, 1990, 3, 475-484.
2. **Biochemical Systematics and Ecology**, 1990, 18, 39-42.
3. **Biochemical Systematics and Ecology**, 1990, 18, 43-44.
4. **Phytochemistry**, 1990, 29, 1193-1195.
5. **Food Additives And Contaminants**, 1990, 7, 303-321.
6. **Biochemical Systematics and Ecology**, 1990, 18, 413-418.
7. **Food Hydrocolloids**, 1991, 5, 297-306.
8. **Biochemical Systematics and Ecology**, 1991, 19, 447-452.
9. **International Tree Crops Journal**, 1991, 6, 275-285.
10. **Food Additives And Contaminants**, 1991, 8, 405-421.
11. **Food Additives And Contaminants**, 1991, 8, 423-436.
12. **International Tree Crops Journal**, 1991, 7, 29-40.

Food Hydrocolloids Vol.3 no.6 pp.475–484, 1990

The characterization of *Acacia paolii* gum and four commercial *Acacia* gums from Kenya

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Abstract. Water-soluble gums from Kenya are reputed to be variable in quality and to differ in functional properties from Sudanese gum arabic, but analytical data for Kenyan gums, capable of explaining such observations, have not been available. The gum from *Acacia paolii* Chiov., a tree of unusual appearance and widespread occurrence in Kenya, has been shown to be dextrorotatory but analytically different from gum talha (*A.seyal*). Of four other Kenyan samples studied, gum from the Lemote district is also dextrorotatory; in contrast, gum samples from Ilaut, Marsabit and Nyoke are laevorotatory but easily distinguishable analytically from classical gum arabic (*A.senegal*) in terms of their specific rotations; viscosities; nitrogen, methoxyl and rhamnose contents; and galactose/arabinose ratios. These analytical differences are reflected in the different efficiencies of these gums as emulsifiers. Fourier-transform ^{13}C-NMR spectra confirm that the gum from *A.paolii* (+90°) differs from Sudanese gum talha (*A.seyal*, +56°) and that gum arabic (*A.senegal*, −30°) differs structurally from the gum from Marsabit. Although measurement of specific rotation distinguishes between gum arabic and gum talha, this simple test is not sufficient to establish that a sample of gum arabic originates solely from *A.senegal* and is therefore likely to have the unique functional properties of that gum essential for manufacturing purposes; for such an assurance, other analytical parameters must also conform to the values established for *A.senegal* gum.

Introduction

A vast area of increasing aridity covers most of northern Kenya: there are also arid areas in the south and east, where the meagre, unreliable rainfall supports only thorny shrubs such as *Acacias* capable of surviving in desert or semi-desert conditions. The latest authoritative botanical text (1) lists 39 *Acacia* species which occur in Kenya: of these, 24 (including *A.seyal*, the main source of commercial gum talha) are members of the *Gummiferae* subsection of the genus and 15 (including *A.senegal*, the defined source of gum arabic for foodstuffs use) are members of the *Vulgares* subsection proposed by Bentham (2) and revised by Vassal (3). Unfortunately, botanical knowledge of the precise identity and characteristics of many of the Kenyan *Acacias* is acknowledged (4) to be incomplete and unsatisfactory, largely as a result of inadequate information concerning the exact nature of the morphological variation in parts of the species' range (4). The need for field observations of the variations in habit, together with notes on the ecological preferences of species, was recorded by Ross (4), who also listed several taxa that cannot be placed with certainty, thus complicating an already confused situation. The known tendency for certain *Acacia* species to continue to hybridize, and the remoteness of some of the arid areas, also contribute to the difficulties involved.

Considerable quantities of *Acacia*-type gums are potentially available in Kenya. The quality of commercial lots offered under the description 'gum arabic', however, has been reputed to be variable and the true identity of the gum frequently questioned. This is now understandable in terms of the botanical

uncertainties and range of species outlined above, in conjunction with the fact that the gum can only be collected from natural, mixed stands of trees because plantations of single species have not been established, in contrast to the long-established practice involving *A.senegal* agroforestry in the Sudan. As analytical data for Kenyan *Acacia* gums have not been available for reference purposes, the opportunity has been taken to study five samples obtained for this purpose.

Materials and methods

Origin of gum samples

A sample described as 'gum arabic' from Marsabit National Park, Northern Kenya, was kindly provided by the UK Overseas Development Natural Resources Institute. An exploratory survey of gum-producing areas in Kenya provided a sample of gum from *Acacia paolii* Chiov. This species, of widespread occurrence, often gregarious and locally dominant (4), yields gum copiously in some locations; it is easily recognized by its characteristically smooth, dark green bark and long pods covered with short, gold-coloured hairs up to 4 mm long. Three other gum samples, characteristic of the dominant *Acacia* spp. in the areas of Lemote, Nyoke and Ilaut, were also obtained for analysis. Only the sample from Marsabit had an external appearance similar to that of Sudanese gum arabic. The sample from Lemote comprised small, friable fragments similar in appearance to gum talha. The samples from *A.paolii*, Ilaut and Nyoke (a very small sample) comprised larger pieces of very pale colour, but of irregular non-descript shapes lacking the characteristic, strongly fissured surface-marking of the gum from *A.senegal.*

Analytical methods

The basic methods used have been described (5). In addition, this report includes assessments of oil-in-water emulsification activity and stability by the method recently proposed (6) in which an Ultraturrax homogenizer is used at 15 000 r.p.m. and the emulsion activity is defined as the absorbance at 500 nm given by a 1 in 250 dilution of an emulsion prepared freshly under precisely defined mixing conditions. The emulsion stability is defined (6) as the absorbance at 500 nm given by a 1 in 250 dilution of the lower half of the emulsion after storage for 30 min, and is expressed as a percentage of the original emulsification activity.

Fourier-transform ^{13}C-NMR spectra for 10% gum solutions in D_2O at room temperature were recorded overnight at 50.32 MHz with either a Brüker WH360 or a Brüker WP200 spectrometer.

Results and discussion

Table I presents the analytical data obtained for the physico-chemical and carbohydrate parameters, and the emulsification data for limonene and hexadecane. Table II shows the amino acid compositions, as residues per 1000 amino acid residues. Table III shows the cation composition expressed as

Table 1. Analytical data for *Acacia paolii* gum and other Kenyan *Acacia* gum samples

	Acacia paolii	Samples from other Kenyan locations				Gum arabic (*A.senegal*)[a]	Gum talha (*A.seyal*)[b]
		Lemote	Nyoke	Ilaut	Marsabit		
Loss on drying (%)	13.8	10.5	17.6	15.8	13.4	13.6	13.4
Total ash, 550°C (%)[c]	1.2	2.2	2.0	2.8	4.6	4.1	2.9
Nitrogen (%)[c]	0.05	0.30	0.12	0.49	0.70	0.33	0.14
Nitrogen conversion factor (Table II)	6.79	6.59	6.81	6.63	6.72	6.60	(6.25)
Hence protein (%)[c]	0.34	2.0	0.81	3.3	4.7	2.2	0.9
Methoxyl (%)[d]	0.30	0.24	0.17	0.12	0.10	0.26	0.94
Tannin (%)	0.3	0.8	0	0.3	0.2	0	1.9
Specific rotation (degrees)[d]	+90	+82	−61	−5	−32	−30	+51
Intrinsic viscosity in 1 mol/dm^3 NaCl (ml/g)[d]	3.7	12	2	19	26	17	12
Neutralization equiv. wt[d]	2300	2130	1080	860	800	1040	1470
Hence uronic anhydride[e]	8	8	16	20	22	17	12
For 25% aq. solutions							
pH	4.3	4.4	n.d.	4.3	4.4	4.4	4.5
Brookfield viscosity (cp)	20	70	n.d.	410	380	90	60
Emulsification activity							
For limonene	1.55	1.54	0.94	2.07	2.10	1.60	0.51
For hexadecane	0.84	0.93	0.47	1.46	1.28	0.79	0.76
Emulsification stability (%)							
For limonene	49	63	43	89	92	95	63
For hexadecane	65	70	47	90	79	70	59
Sugar composition after hydrolysis							
4-*O*-Methylglucuronic acid[f]	2	1.5	1	1	0.5	1.5	5
Glucuronic acid	6	6.5	15	19	20.5	15.5	7
Galactose	33	38	61	55	54	46	38
Arabinose	55	54	17	16	16	24	46
Rhamnose	4	<1	6	9	8	13	4

[a] From ref. 21.
[b] From ref. 5.
[c] Corrected for loss on drying.
[d] Corrected for loss on drying and protein content.
[e] If all acidity arises from uronic acids.
[f] If all methoxyl content located in this acid.

Table 2. Amino acid composition (residues per 1000 residues) for *Acacia paolii* gum and other Kenyan *Acacia* gum samples

	Acacia paolii	Samples from other Kenyan locations				Gum arabic (*A.senegal*)[a]
		Lemote	Nyoke	Ilaut	Marsabit	
Alanine	61	49	45	33	25	28
Arginine	9	0	3	0	0	5
Aspartic acid	94	85	63	46	39	50
Cystine	0	0	0	0	0	0
Glutamic acid	44	45	35	30	22	29
Glycine	51	50	33	46	38	41
Histidine	28	61	42	56	37	44
Hydroxyproline	245	252	320	322	456	328
Isoleucine	29	23	16	11	0	12
Leucine	65	57	59	70	47	67
Lysine	21	22	11	15	0	23
Methionine	0	0	0	0	0	1
Phenylalanine	25	21	17	18	11	22
Proline	59	87	98	72	138	88
Serine	112	113	147	157	100	136
Threonine	57	55	51	84	65	76
Tyrosine	13	13	11	4	2	10
Valine	88	65	49	36	20	36
Hence nitrogen conversion factor	6.79	6.59	6.81	6.63	7.01	6.60

[a]From ref. 22.

Table 3. The cationic composition (μg/g) of the ash from *Acacia paolii* and other Kenyan *Acacia* gum samples

	Acacia paolii	Gum samples from other Kenyan locations				Gum talha (*A.seyal*)[a]	Gum arabic (*A.senegal*)
		Lemote	Nyoke	Ilaut	Marsabit		
% ash	1.2	2.2	2.0	2.8	4.6	4.2	3.9
Aluminium	ND	ND	ND	ND	ND	6100	171
Calcium	302 000	300 000	324 000	356 000	219 000	260 380	235 370
Cadmium	0	0	0	0	0	0	0
Chromium	0	0	0	4	8	40	49
Cobalt	495	465	466	193	0	24	0
Copper	0	0	0	10	46	55	29
Iron	612	883	560	147	1120	2860	105
Potassium	120 000	107 000	193 000	126 000	59 600	47 760	193 700
Magnesium	39 800	36 500	79 900	23 100	29 200	27 910	48 250
Manganese	52	57	0	0	60	49	221
Nickel	0	0	25	4	0	10	5
Lead	12	7	8	0	17	4	3
Sodium	13 000	13 000	520	1360	1740	2180	780
Zinc	76	203	8	4	73	23	10

[a]Data from ref. 5.

micrograms per gram of the ash obtained at 550°C from the gum samples. Data for *A.senegal* and *A.seyal* are included for comparative purposes in Tables I–III.

Figure 1 shows Fourier-transform ^{13}C-NMR spectra obtained for the gums from *A.paolii* and *A.seyal*; Figure 2 shows spectra for the gums from *A.senegal* and from Marsabit.

Of the five samples studied, clear distinctions can be made between those from *A.paolii* and from Lemote, which are dextrorotatory, and those from Nyoke, Ilaut and Marsabit, which are laevorotatory.

Acacia paolii, a globose-flowered species with large spinescent stipules in pairs, is a member of the subsection *Gummiferae* (2) and related to *A.seyal*, the major Sudanese source of gum talha. It occurs extensively in Northern Frontier Province (4), where it is also known (4) to hybridize with *A.nubica* Benth., which gives gum with one of the most highly positive specific rotations recorded (7). Analytically, the gums from *A.paolii* and Lemote show (Table I) similarities (tannin content, low rhamnose content, poor emulsification power) to gum talha (*A.seyal*), yet differ clearly from it in several ways (different nitrogen and methoxyl contents; lower uronic acid content; higher ratios of arabinose to galactose). The very low rhamnose content of Lemote gum is not exceptional: rhamnose contents of 1% or less have been recorded for the gums from *A.gerrardii* (8) and *A.drepanolobium* (9), which are common in East Africa, and for *A.calcigera* gum (10). The sugar composition of the latter two species is very similar to that of the gum from Lemote but *A.drepanolobium* gum has a strong tendency to give gels rather than solutions (9).

In terms of their amino acid compositions, the two Kenyan dextrorotatory gums do not show (Table II) values differing greatly from those for other East African *Acacia* spp. (5). In contrast, their cationic compositions show unusually

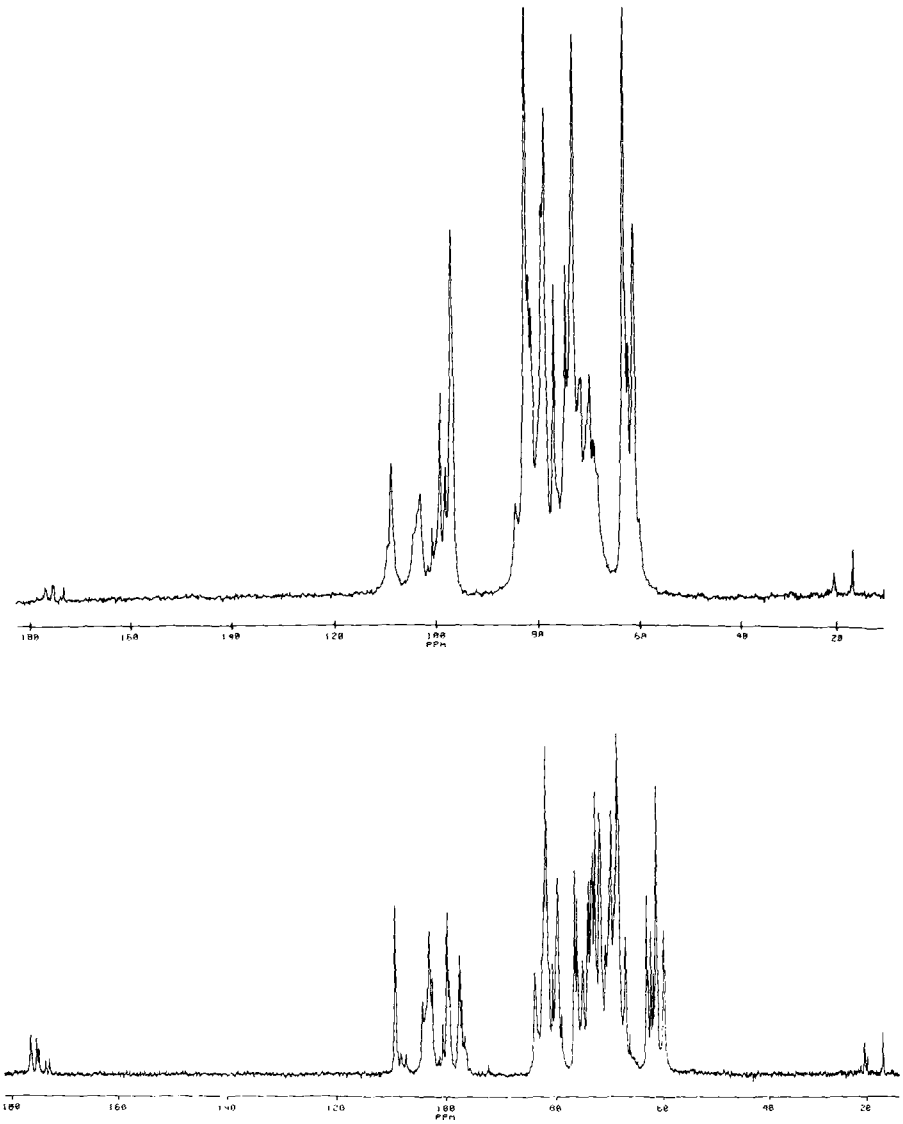

Fig. 1. ^{13}C-NMR spectra for gum from *A.paolii* (top) and *A.seyal* (bottom).

high cobalt contents and lower chromium and copper contents (Table III) than have been found in East African species (5), presumably reflecting the abundance of elements in soils at different locations. Confirmation of structural differences between the gums from *A.paolii* and *A.seyal* was given by Fourier-transform NMR spectroscopy (Figure 1); assignments of resonances are given below.

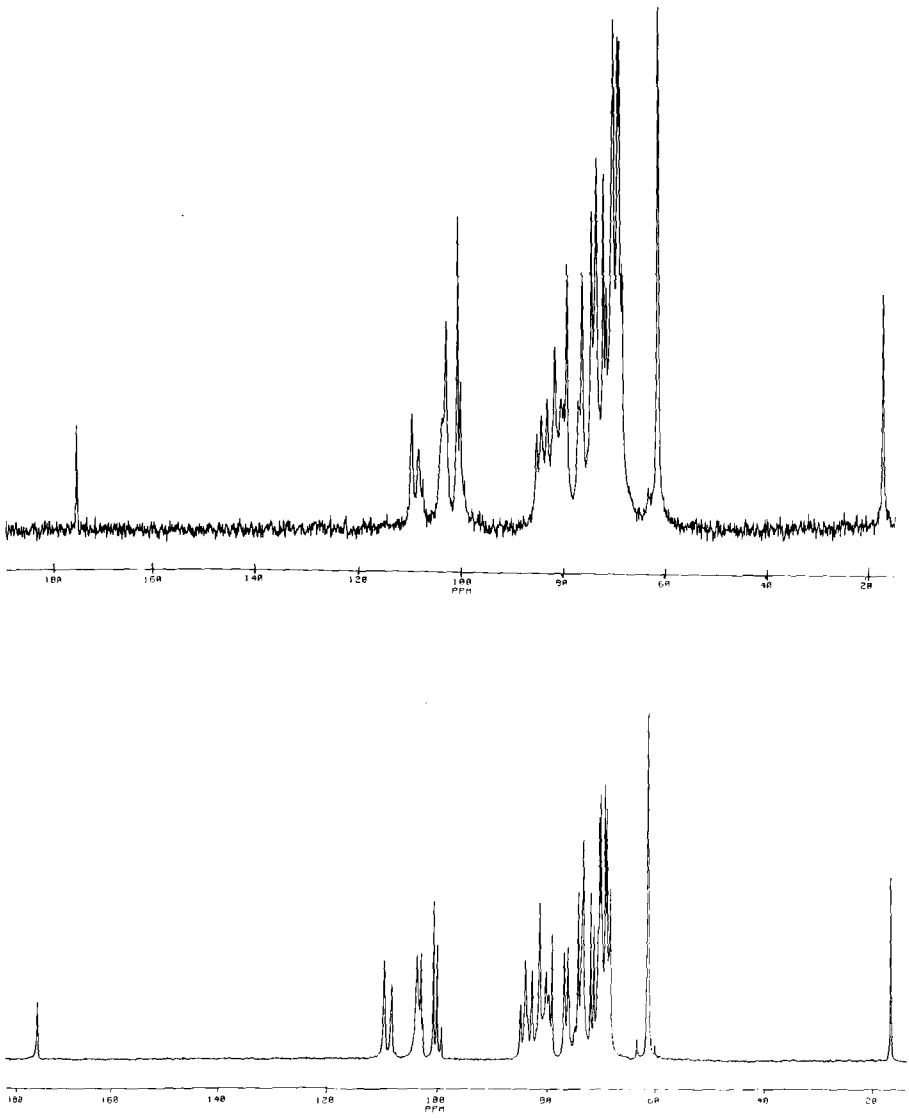

Fig. 2. ^{13}C-NMR spectra for gum from Marsabit (top) and *A.senegal* (bottom).

The three laevorotatory commercial samples, from Nyoke, Ilaut and Marsabit, show extensive analytical differences between themselves, and they also differ clearly from Sudanese gum arabic derived from *A.senegal* (Table I). Thus, for these four gums, nitrogen content varies from 0.12 to 0.70%; methoxyl content varies from 0.10 to 0.26%; specific rotation from −5° to −61°; intrinsic viscosity from 2 to 26 ml/g; rhamnose content from 6 to 13%; uronic acid content from 16 to 22%; ratios of arabinose/galactose from 17/61 to 24/46; and

emulsification stability (6) from 43 to 95% for limonene and from 47 to 90% for hexadecane. It has long been known (11) that the emulsification processes involved are complex, and that small initial emulsion droplet size may be misleading (12) in terms of long term stability. It has also been established (12) that the emulsifying properties of gum samples from different *Acacia* species is dependent not only on their peptide/protein content but also on their distribution and availability for adsorption. Although James and Patel have claimed (6) that no standard emulsification test exists, their procedure suffers from the deficiencies of previous procedures involving turbidity measurement at a single wavelength (13) after a mixing (as opposed to true emulsification) procedure, as discussed critically by Dickinson and Stainsby (14). Nevertheless, a mixing method has recently been used meaningfully (15) to assess the stability of emulsions. The rapid method of assessment (6) used here not only gave reproducible results (±3%) but also confirmed the relative emulsion efficiencies found (12) for the six gum samples from different *Acacia* species used in a much more detailed, fundamental study.

Of the three laevorotatory gums studied here, those from Ilaut and Marsabit contain small proportions of tannin (Table I) which are not permitted in food grade gum arabic. The cation composition (Table III) of the gum from Marsabit differs from those from Nyoke and Ilaut in containing no cobalt but much higher iron, manganese, lead and zinc contents. The amino acid compositions of the gums from Nyoke and Ilaut do not differ greatly from the values established for *A.senegal* (Table II). The gum from Marsabit, however, has exceptional features in addition to its high nitrogen content (0.70%). It contains very high proportions of hydroxyproline and proline, which together account for 60% of all the amino acids present. Correspondingly, there are smaller amounts of all of the other amino acids present; the absence of isoleucine and lysine has not been observed previously. These unusual proportions of amino acids are reflected in an unusually high value for the nitrogen conversion factor (Table II).

The major interest, therefore, lies in the other analytical differences between the gums from Marsabit and *Acacia senegal* (Table I) despite the closeness of their specific rotations. Major differences include the low rhamnose and methoxyl contents; the very high nitrogen, uronic acid and galactose/arabinose ratio; and, in particular, the very high viscosity of the gum from Marsabit. (Sudanese gum arabic from *A.senegal* gives an average value of 90 cp with the range extending from 60 to 120 cp for 25% (w/v) aqueous solutions.)

Confirmation that the analytical differences between the gums from *A.senegal* and from Marsabit reflect structural differences was obtained by Fourier-transform ^{13}C-NMR spectra (Figure 2). The spectrum for *A.senegal* gum (Figure 2) confirms that published by Artaud *et al.* (16), who also established that different samples of gum arabic from *A.senegal* (syn. *verek*) gave superimposable spectra. The following Fourier-transform ^{13}C-NMR assignments have long been established (16): C-methyl groups in rhamnose (16–17 p.p.m.); $-CH_2$ groups at C6 of hexoses (60–62 p.p.m.); –CH groups at C2–C5 of hexoses (65–85 p.p.m.); anomeric –CH groups at C1 (95–105 p.p.m.); and the carbonyl resonance in C6 uronic acid groups (174–176 p.p.m.)

The botanical deficiencies (17) in the identification of the 13 members of the *Acacia senegal* complex (18) have received comment. The literature (1,4) indicates that the members of the *A.senegal* (L.) Willd. complex endemic in Kenya include *A.condyloclada* Chiov., *A.hamulosa* Benth. and *A.thomasii* Harms. In addition, several of the varieties of *A.senegal* are known to occur in Kenya, e.g. var. *senegal*, var. *kerensis* Schweinf., var. *rostrata* Brenan and var. *leiorachis* Brenan. Furthermore, the latter variety embraces two different forms in Kenya (4): in addition to a straggling growth form reminiscent of *A.thomasii*, there is a non-virgate branching form that grows into a substantial tree with a rounded crown, to which the name *A.circummarginata* Chiov. was assigned. Clearly there is a great need for botanical voucher reference specimens of leaves, pods, inflorescences, etc. to be collected next season from the dominant species in the Nyoke, Ilaut and Marsabit areas to permit their positive identification, as has been achieved this season for *A.paolii*. Steps are being taken to achieve this and to extend surveys to other districts as a contribution to the field studies in north-east tropical Africa declared by Brenan (17) and Ross (18) to be essential to clarify the taxonomic problems encountered with the *A.senegal* complex. Regrettably, no positive response appears to have been taken to date. Analytical data for gum exudates can provide a sensitive way of resolving taxonomic problems (19,20).

Conclusions

The ubiquitous *A.paolii* and other dextrorotatory species in the Lemote area are sources of gums that are similar in type to, but structurally different from, *A.seyal*, the major source of Sudanese gum talha. Measurement of specific rotation remains adequate to distinguish these gum talha-like samples from authentic laevorotatory gum arabic. Although the gums from the Nyoke, Ilaut and Marsabit areas are laevorotatory, they are analytically and functionally different from classical Sudanese gum arabic derived from *A.senegal* (L.) Willd. The simple measurement of specific rotation would again be adequate to indicate the difference in identity and hence performance of the gums from Nyoke and Ilaut, but not for the gum from Marsabit which, fortunately, can be distinguished from *A.senegal* gum analytically if data for its nitrogen, methoxyl, rhamnose and tannin contents, and intrinsic viscosity, are also obtained. Without such attention to analytical detail, however, samples described indiscriminately as 'gum arabic' without any specification must be suspected to be of variable identity, functionality and value. It is foolhardy to base decisions on external appearance alone if gum of a specific performance is required.

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The Composition and Properties of Eight Gum Exudates (Leguminosae) of American Origin

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Key Word Index—*Cassia*; *Caesalpinia*; *Cercidium*; *Lysiloma*; *Senna*; *Enterolobium*; *Parkia*; Leguminoisae; gum exudates; amino acids; chemotaxonomy.

Abstract—Analytical data are presented for the polysaccharide and proteinaceous components of American samples of the gum exudates from *Cassia grandis, Caesalpinia eriostachys* and *Caesalpinia* sp. *nov., Cercidium praecox, Lysiloma acapulcense, Senna nicaraguensis, Enterolobium cyclocarpum* and from the gum extracted from the seed pods of *Parkia nitida*, all of which are members of the family Leguminosae. The gums from *Cassia grandis* and *Cercidium praecox* are very acidic; *Cassia grandis* gum contains an unusually high proportion of glycine; *Cercidium praecox* gum has a high nitrogen content; and the gums from *Senna nicaraguensis* and *Caesalpinia* sp. *nov.* are very viscous. All of the samples contain tannin; none are permitted in foodstuffs. The data may be of taxonomic interest; the gums from *Cassia grandis, Caesalpinia* sp. *nov.* and *Cercidium praecox* differ from the others studied in containing major amounts of galacturonic acid and xylose.

Introduction

Analytical data are now available for many African and Australian samples of the gum exudates from leguminous species, particularly for the genera *Acacia*, (of which *ca* 120 species have been studied [1, 2]) and *Albizia* [3, 4]. Because few American leguminous species, apart from *Prosopis* [5, 6], have been studied to date, the opportunity has been taken to characterize eight American gum samples which became available recently.

Results

The analytical data for the physico-chemical and carbohydrate parameters are shown in Table 1; for the amino acid composition in Table 2; and for the cationic composition of the ash in Table 3.

Discussion

Four of the gums studied (*Cassia grandis, Enterolobium cyclocarpum, Senna nicaraguensis* and *Caesalpinia eriostachys*) were only *ca* 50% soluble in cold water; the other four gum samples had excellent solubility.

In 1982, Irwin and Barneby [7] divided neotropical *Cassia* into three genera, *viz. Cassia sensu stricto, Senna* and *Chamaecrista*; Lock [8] subsequently made the necessary combinations for the African *Senna* and *Chamaecrista* spp. As a result, only about 15 or 16 *Cassia* (*sensu stricto*) spp. are recognised in the neotropics. No data for gum exudates from *Cassia* or *Senna* spp. appear to have been published previously, but this study has shown that the gums from *Cassia grandis* and *Senna nicaraguensis* (Table 1) differ significantly. Thus, *Senna nicaraguensis* gum has a very high viscosity, a high proline content (Table 2) and contains glucuronic acid, galactose and arabinose; *Cassia grandis* gum is of low viscosity (Table 1), contains galacturonic acid, galactose, arabinose and xylose, and contains, in addition, a very large proportion of glycine and hence has a very low nitrogen conversion factor (Table 2). Glycine usually occurs to the extent of only 50–150 residues per thousand residues in most exudate gums, although a higher value (249 residues per 1000 residues) was recorded [9] for a low-viscosity sample of a seed galactomannan (guar gum). *Cassia grandis* gum contains amounts of manganese and zinc (Table 3) which must be regarded as high for a gum exudate.

Enterolobium is a small genus (*ca* 7 or 8 species); members are native to tropical America

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TABLE 1. ANALYTICAL DATA FOR EIGHT GUM SAMPLES, FAMILY LEGUMINOSAE

	Cassia grandis	*Caesalpinia* sp. *nov.*	*Cercidium praecox*	*Lysiloma acapulcense*	*Senna nicaraguensis*	*Caesalpinia eriostachys*	*Enterolobium cyclocarpum*	*Parkia nitida*
Loss on drying (%)	16.1	14.1	12.7	14.1	17.5	18.1	11.6	13.3
Total ash, 550°C (%)	15	4.6	4.8	3.2	3.3	3.0	6.9	2.1
Nitrogen (%)	0.63	0.66	1.8	0.30	0.44	0.25	0.26	0.37
Nitrogen conversion factor (Table 2)	5.15	5.87	5.93	6.47	6.33	6.07	6.31	6.83
Hence protein (%)	3.2	3.8	10.6	1.9	2.8	1.5	1.6	2.5
Methoxyl (%)	2.4	0.8	1.2	0.6	1.2	1.8	1.0	0.15
Tannin (%)	0.3	0.15	0.22	0.9	0.27	0.25	0.25	0.36
Specific rotation (degrees)	+82	+10	−11	−2	+5	−15	+40	−35
Intrinsic viscosity (ml/g)	10	190	34	5	216	16	67	7
Neutralization equiv. wt	400	625	524	1240	814	1114	950	1240
Hence, uronic anhydride	44	28	34	14	22	16	19	14
Sugar composition after hydrolysis								
4-*O*-Methylglucuronic acid	0	0	0	3.5	7	11	6	1
Glucuronic acid	0	0	0	10.5	15	5	13	13
Galacturonic acid	44	28	34	0	0	0	0	0
Galactose	30	35	29	69	43	45	37	43
Arabinose	10	29	5	13	35	39	32	39
Xylose	16	8	32	0	0	0	0	0
Rhamnose	0	0	0	4	0	0	12	4

TABLE 2. AMINO ACID COMPOSITION (RESIDUE PER 1000 RESIDUES) FOR EIGHT GUM SAMPLES, FAMILY LEGUMINOSAE

	Cassia grandis	*Caesalpinia* sp. *nov.*	*Cercidium praecox*	*Lysiloma acapulcense*	*Senna nicaraguensis*	*Caesalpinia eriostachys*	*Enterolobium cyclocarpum*	*Parkia nitida*
% N	0.63	0.66	1.80	0.30	0.44	0.25	0.26	0.37
Alanine	32	108	129	74	120	123	70	89
Arginine	8	16	0	0	0	0	0	0
Aspartic acid	109	111	108	78	80	100	128	112
Cystine	0	0	0	0	0	0	0	0
Glutamic acid	75	81	61	55	59	78	59	61
Glycine	464	119	56	38	81	100	82	56
Histidine	37	47	46	62	31	69	48	32
Hydroxyproline	68	41	80	207	28	37	86	174
Isoleucine	7	42	40	0	34	34	34	36
Leucine	13	55	77	37	56	60	54	56
Lysine	37	104	56	34	72	60	86	27
Methionine	1	0	31	0	0	0	0	0
Phenylalanine	13	22	45	27	24	27	12	21
Proline	4	65	46	99	201	86	121	78
Serine	68	47	70	152	84	67	75	119
Threonine	18	42	71	44	46	54	49	53
Tyrosine	29	11	23	16	25	21	31	31
Valine	17	89	61	77	59	84	65	55
Hence, nitrogen conversion factor	5.15	5.87	5.93	6.47	6.33	6.07	6.31	6.83

and the West Indies [10]. *E. cyclocarpum*, one of the best known species, is a fast-growing, large tree known as "elephant's ear" or as "guanacaste" in Costa Rica: its jelly-like exudate gum ("gum de caro") has been reported to be used in folk medicinal treatments [10]. This tree has been recommended for more extensive propagation; it can survive on very dry sites [11]. Its gum has a composition and properties similar to those of the dextro-rotatory, tannin-containing *Acacia* gums of the gum talha type [2]; unfortunately, its poor solubility decreases the number of local technological applications in which it can be used to replace imported gums. This gum contains exceptionally low and high proportions (Table 2) of calcium and potassium, respectively.

Parkia oppositifolia Spruce ex Benth. has been placed in synonymy under *Parkia nitida* Miquel [12]; it is found abundantly in the Amazon region. When extracted with water, its pods gave the gum studied here in 13.5% yield. This gum has been found [13] to be an excellent replace-

TABLE 3. THE CATIONIC COMPOSITION OF THE ASH FROM EIGHT GUM SAMPLES, FAMILY LEGUMINOSAE

	Cassia grandis	*Caesalpinia* sp. *nov.*	*Cercidium praecox*	*Lysiloma acapulcense*	*Senna nicaraguensis*	*Caesalpinia eriostachys*	*Enterolobium cyclocarpum*	*Parkia nitida*
% Ash	15	4.6	3.3	3.0	3.2	4.8	6.9	2.1
Calcium	244,000	236,000	314,000	221,000	43,700	121,000	8800	35,600
Cadmium	0	0	0	0	0	0	0	22
Chromium	<5	15	34	27	24	11	<5	16
Cobalt	<5	0	0	0	0	0	<5	40
Copper	19	0	0	0	0	0	21	81
Iron	350	87	137	852	277	76	225	705
Lead	0	0	0	168	0	0	0	16
Magnesium	41,370	14,700	60,500	18,500	23,500	64,700	10,170	107,000
Manganese	1010	192	123	293	200	315	0	360
Nickel	0	0	0	38	0	0	2	3
Potassium	183,700	193,000	35,400	201,000	261,000	126,000	468,750	121,000
Sodium	3450	2790	317	3460	200	1980	3230	750
Zinc	250	0	0	127	0	0	0	53

ment for gum arabic in applications involving the pelleting and inoculation of legume seeds with *Rhizobium* to assist rapid nodulation. A previous study [14] compared the properties and composition of the comparable seed-pod gum from *Parkia pendula* (Willd.) Benth. ex Walp. with the exudate gums from *P. bicolor* A. Chev. and *P. biglobosa* (Jacq.) R.Br. ex G. Don. It is now apparent that there are similarities between the seed-pod gums from *P. pendula* [14] and *P. nitida* (Table 1); both are laevorotatory and have very similar nitrogen contents, but the intrinsic viscosity of *P. pendula* seed-pod gum (34 ml/g) is considerably greater than that of the exudate gum arabic (16 ml/g) from *Acacia senegal* (L.) Willd. and of the seed-pod gum from *P. nitida* (7 ml/g; Table 1). The gum extractable from the seed-pods of other *Parkia* species may therefore also serve as pelleting adhesives in other locations. The importation of food grade gum arabic from the Sahelian Zone in Africa would be unjustifiable economically for such a purpose. *Parkia nitida* gum contains a comparatively high amount of magnesium (Table 3).

Lysiloma, with *ca* 35 species, is a Mexican genus with extensions into Central America and the West Indies. Extractions of the bark of various species are used for tanning [10]; the gum exudate from *Lysiloma acapulcense* studied here has a high tannin content (Table 1). It also has a very low intrinsic viscosity, but its other analytical parameters are similar to those for gums from other leguminous genera except for comparatively high contents of serine (Table 2) and lead (Table 3).

Caesalpinia is a large genus of ancient origin. The Argentinian species *C. praecox* R. and P. [the basionym of *Cercidium praecox* (R. and P.) Harms] exudes "gum brea" which has been reported [10] to be an acceptable substitute for gum arabic in adhesives and in textile and other native applications [15]. Of the two *Caesalpinia* species studied here, the gum from *C. eriostachys* has a sugar composition that is devoid of rhamnose. It therefore has similarities to that of *Senna nicaraguensis* gum (Table 1) and is distinctly different from the gum from *Caesalpinia* sp. *nov.*, which is very viscous, contains galacturonic acid and xylose, and can therefore be regarded as having a closer affinity with the gums from *Cassia grandis* and *Cercidium praecox*.

Cercidium ("palo verde") is a small genus, widespread in tropical and sub-tropical America, and well-adapted to arid and semi-arid areas [10]. *Cercidium* was formerly congeneric with *Parkinsonia*; these two genera may eventually be re-united, as intrageneric hybrids are known. The gum from *Cercidium praecox*, reported to have local uses in soap-making [16], has high methoxyl and nitrogen contents, fairly high viscosity (Table 1), and a high methionine content (Table 2). Its most interesting features, however, are the very high contents of xylose and galacturonic acid, as has also been found here in the gums from *Cassia grandis* and *Caesalpinia* sp. *nov.*

The gums from *Cercidium praecox, Cassia grandis* and *Caesalpinia* sp. *nov.* therefore differ from the majority of the Leguminosae gums

studied to date, comprising approx. 150 species in all from *Acacia* [1, 2], *Albizia* [3, 4], *Prosopis* [5, 6], *Parkia* [14], *Entada* [17] and *Leucaena* [18, 19]. Gums from these genera are based on proteinaceous polysaccharides containing various proportions of glucuronic and 4-*O*-methylglucuronic acids, galactose, arabinose and rhamnose. Gums from some leguminous genera (e.g. *Brachystegia* and *Julbernardia*), however, contain both glucuronic and galacturonic acids [20] as do gums from the *Combretaceae* [21]. In contrast, the exudates from the leguminous genus *Astragalus* contain only galacturonic acid but have a more complex mixture of neutral sugars, including fucose, a common component of marine algae [22, 23]. Such divergences are not surprising within a Family as large as the Leguminosae, which comprises some 17,500 species in about 670–700 genera with a worldwide distribution, but they may indicate affinities which may not be readily detectable from external morphological characteristics alone. The possible chemotaxonomic importance of gum exudates and other secondary products [24] has long been recognised [25] and utilized [26].

Experimental

Origin of gum samples. Gum extracted from the seed-pods of *Parkia nitida* Miquel was sent for analysis by Avilio A. Franco, Programa Nacional de Pesquisa em Biologia do Solo, Sèropedica, Rio de Janeiro. The exudate gums from *Cassia grandis* L.f. and *Enterolobum cyclocarpum* (Jacq.) Griseb. were collected in Costa Rica by Professor D. H. Janzen, University of Philadelphia. The following exudate gum samples were collected by G. P. Lewis and C. E. Hughes: *Cercidium praecox* (Ruiz. and Pav.) Harms from Puebla, Mexico and *Caesalpinia* sp. *nov.* from Oaxaca, Mexico; *Lysiloma acapulcense* (Kunth) Benth. and *Caesalpinia eriostachys* Benth. from Dept Santa Ana, El Salvador; *Senna nicaraguensis* (Benth.) Irwin and Barneby from Dept Santa Rosa, Guatemala.

Analytical methods. The standard analytical methods used have been described [2].

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The Composition of Some *Sesbania* Gum Exudates

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Key Word Index—*Sesbania*; Leguminosae, gum exudates; amino acids; arabinogalactans.

Abstract—An analytical study has been made of gum exudates from four specimens of *Sesbania sesban* (Family Leguminosae); *Sesbania sesban* var. *nubica* and *Sesbania grandiflora*. They are strongly dextrorotatory, acidic arabinogalactans and give strongly coloured solution of low viscosity comparable to gum talha (*Acacia seyal*).

Introduction

There have been references in earlier literature [1–4] to the potential value of *Sesbania* gums for industrial purposes, but analytical data have not been available for them. Moreover, the reports did not differentiate between the exudate gums and the seed galactomannan gums from *Sesbania* species. Increased attention is now being given to exploiting the potential of *Sesbania* species for a range of purposes, as recommended by the U.S. Academy of Sciences [1, 3]. The opportunity has, therefore, been taken to study samples from Kenya and Hawaii of the gum exudates from *Sesbania sesban* (four specimens), *S. sesban* var. *nubica* and *S. grandiflora*.

Results and Discussion

The analytical data obtained are shown in Tables 1 and 2, which include data for gum talha and gum arabic for comparative purposes. The six samples studied are all strongly dextrorotatory; previously, the highest specific rotation recorded [5] for a gum exudate was that for *Acacia nilotica* (+108°). None of the *Sesbania* gum samples contained more than a trace of rhamnose and all gave dark coloured solutions of low viscosity. The major differences occurred between the two Hawaiian samples, with the gum from *S. sesban* var. *nubica* being considerably more proteinaceous, but much less viscous than the other gum samples. Of the four Kenyan samples from *S. sesban*, that from Afrena was considerably more acidic than the others and that from Maseno was less nitrogenous. As now established for gum exudates from a wide range of genera [e.g. 6], these *Sesbania* gum exudates contain amino acids, as shown in Table 2. Gum exudates from genera within the *Leguminosae* usually contain very large proportions of hydroxyproline [7] but this is not the case for *S. sesban* nor for *S. sesban* var. *nubica* gums.

The very dark brown or red solutions given by these gums, reported previously [2, 4], limits their use in commercial applications other than possibly for small-scale native uses. Claims [1, 2] that *Sesbania* gums may be used as a substitute for gum arabic must be questioned; the *Sesbania* gums studied have similarities to commercial gum talha (*Acacia seyal*) but not to gum arabic [*Acacia senegal* (L.) Willd.] (Table 1). The *Sesbania* gums are not included in any of the regulatory permitted lists of food additives; no toxicological evaluations of their safety exists. At this stage it therefore appears that *Sesbania* gum exudates are unlikely to meet widespread commercial demand, despite indications to the contrary [2, 4], and development programmes should take this into account. Large quantities of industrial (i.e. non-food) gum exudates from *Combretum* and other genera are already available at low prices.

In contrast to the perennial *Sesbania* gum exudates, the seed endosperm galactomannan gums from annual *Sesbania* species are well known [8], characterized chemically [9–11], and

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TABLE 1. COMPARATIVE DATA FOR SIX *SESBANIA* GUM SAMPLES, GUM ARABIC AND GUM TALHA

	Sesbania sesban var. *nubica* (Hawaii)	*Sesbania grandiflora* (Hawaii)	*Sesbania sesban* (Kenya) ex Maseno	ex Afreno	ex Yala	ex Kima	*Acacia seyal** (gum talha)	*Acacia senegal*† (gum arabic)
Nitrogen (%)	0.63	0.15	0.05	0.13	0.16	0.17	0.14	0.31
Nitrogen conversion factor (see Table 2)	6.56	(6.25)	(6.25)	(6.25)	(6.25)	6.57	(6.25)	6.63
Hence, protein (%)	4.1	0.9	0.3	0.8	1.1	1.1	0.9	2.0
Specific rotation (degrees)	+96	+159	+120	+128	+109	+90	+51	−30
Intrinsic viscosity (ml/g)	2	16	12	8	10	9	12	17
Neutralization equivalent weight (g)	980	1990	1410	860	1320	1410	1470	1020
Hence, uronic acid (%)	18	9	12	20	13	12	12	17
Neutral sugar composition (%) after acid hydrolysis								
Galactose	45	53	56	43	59	42	38	45
Arabinose	36	38	32	37	28	46	46	24
Rhamnose	tr	tr	tr	tr	tr	tr	4	14

†From ref. [14]; *from ref. [13].

TABLE 2. THE AMINO ACID COMPOSITION (RESIDUES PER 1000 RESIDUES) OF THE PROTEIN/PEPTIDE COMPONENT OF SOME *SESBANIA* AND *ACACIA* GUM EXUDATES

	Sesbania sesban var. *nubica*	*Sesban* ex Yala	*Acacia** *senegal*
%N	0.63	0.16	0.35
Alanine	53	59	31
Arginine	28	32	7
Aspartic acid	242	219	60
Cystine	29	79	1
Glutamic acid	80	99	36
Glycine	71	81	49
Histidine	14	18	51
Hydroxyproline	0	13	274
Isoleucine	22	47	14
Leucine	27	49	75
Lysine	25	38	26
Methionine	7	24	0
Phenylalanine	19	25	29
Proline	262	49	77
Serine	45	60	137
Threonine	24	36	77
Tyrosine	14	23	11
Valine	36	50	45
Hence, nitrogen conversion factor	6.56	6.57	6.60

*From ref. [15].

established commercially although they too are not permitted in foodstuffs.

Experimental

Origin of gum specimens. Gum from *Sesbania sesban* (L.) Merr. var. *nubica* (Chiov.) was collected in Hawaii by Mr Bob Wheeler in 1988, and gum from *Sesbania grandiflora* was collected at Waimanolo, Hawaii by Mr Bill Macklin in February 1989. Gum from *Sesbania sesban* (L.) Merr. was collected by field staff of ICRAF, Nairobi, in Western Kenya in January 1989; small gum specimens (each of only 1 or 2 g) were obtained from four location, *viz* Maseno, Kakamega in Afrena, Yala and Kima.

Analytical methods. The standard methods for the analysis of gum exudates, including amino acid analyses, have been described [12].

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COMPOSITION OF THE GUM FROM *COMBRETUM PANICULATUM* AND FOUR OTHER GUMS WHICH ARE NOT PERMITTED FOOD ADDITIVES

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(*Received* 30 *August* 1989)

Key Word Index—*Combretum*; *Pseudocedrela*; Meliaceae; *Sclerocarya*; Anacardiaceae; *Cassine*; Celastraceae; *Atalaya*; Sapindaceae; sugar composition; amino acid composition; gum exudate.

Abstract—Only three gum exudates are permitted for pharmaceutical and food use by international regulatory authorities, viz. gum tragacanth (Asiatic *Astragalus* spp.), gum karaya (*Sterculia* spp.) and gum arabic [*Acacia senegal* (L.) Willd.], but a wide range of other tree exudates is used for a variety of uses in their countries of origin. This paper presents analytical data for the gum exudates from *Atalaya hemiglauca*, *Cassine aethiopica*, *Combretum paniculatum*, *Sclerocarya birrea*, and *Pseudocedrela kotschyi*. These gums may have local technological applications, but are not recommended for addition to foodstuffs.

INTRODUCTION

Because modern food and pharmaceutical regulations prohibit their use, the gum exudates from many *Combretum* [1, 2], *Grevilleae* [3], *Leucaena* [4], *Prosopis* [5], *Albizia* [6] and non-permitted *Acacia* spp. [7] have been characterized to facilitate their detection in commercial gum consignments. Nevertheless, a wide range of trees from these genera are recorded [8, 9] as having traditional, food and other uses within the local environments where the trees occur abundantly. This report presents analytical data for the gums exuded by *Atalaya hemiglauca*, *Cassine aethiopica*, *Sclerocarya birrea*, *Pseudocedrela kotschyi* and *Combretum paniculatum*. These gums have not been investigated previously.

RESULTS

The analytical data for sugar compositions and physico-chemical parameters are shown in Table 1; for amino acid compositions (residues per 1000 amino acid residues) in Table 2; and for cation compositions of the ash obtained from the gums at 550° (μg per gram of ash) in Table 3.

DISCUSSION

All of these gum samples dissolved readily in cold water. The gum solutions from *Sclerocarya birrea* and *Pseudocedrela kotschyi* were dark brown in colour, the poor quality of these gums has already been noted [10].

Combretum paniculatum Vent. and *Combretum microphyllum* Klotsch. are very closely related, with no convincing way available to separate them in the Herbarium although fieldsmen appear to be convinced that they differ [11]. They are found in wetter regions of tropical Africa. The *Combretum* gums are the most frequently occurring adulterants of commercial gum arabic [*Acacia senegal* (L.) Wild.]. The sugar and amino acid compositions of *C. paniculatum* gum are typical of the dextrorotatory *Combretum* gums [2] having low nitrogen and high rhamnose contents and giving viscous solutions. Its methoxyl content is high (Table 1) and it has a high zinc content (Table 2). Its amino acid composition shows the features already established [2] for the *Combretum* gums viz. high in aspartic acid and glycine, relatively low in hydroxyproline. The tannin content will give this gum an astringent taste, the *Combretum* gums are not permitted in foodstuffs and its use in such, in any location, should be avoided.

The gum from *Atalaya hemiglauca* is of interest in having a negative specific rotation very close to that of gum arabic from *Acacia senegal*, which can be regarded as having a specific rotation of $-30 \pm 3°$ [12]. Care to differentiate between these two gums may therefore be necessary, although Australian exudates are not usually exported. Fortunately, clear distinctions can be made on the basis of large differences in several of the other analytical parameters, e.g. the very low nitrogen, rhamnose and viscosity, the high methoxyl content, and the tannin content in *Atalaya hemiglauca* gum (Table 1). Its amino acid composition also differs greatly from that of gum arabic [13], key differences being the low hydroxyproline and high aspartic acid and cystine content of the *Atalaya* gum (Table 2). A higher cystine content was reported [14] for *Grevillea robusta* gum.

Cassine aethiopica is widespread in tropical Africa [15]. Its gum has very low, but not exceptional, nitrogen, rhamnose and methoxyl contents (Table 1) and has a fairly low hydroxyproline content [14]. It has features which are comparable to those of gum talha (*Acacia seyal*).

The gum from *Pseudocedrela kotschyi* is reported [8] to be used medicinally by natives in West Africa and as an arrow poison. It has a very low arabinose and rhamnose content and its amino acid composition has features (high

Table 1. Analytical data for five gum exudates

	Atalaya hemiglauca	*Cassine aethiopica*	*Combretum paniculatum*	*Pseudocedrela kotschyi*	*Sclerocarya birrea*
Loss on drying, 100°C (%)	15.2	10.7	11.5	13.8	13.1
Total ash, 550°C (%)*	3.2	0.5	3.5	4.1	7.1
Nitrogen (%)*	0.21	0.08	0.05	0.13	0.16
Nitrogen conversion factor†	6.47	6.49	6.31	6.75	6.77
Hence protein (%)	1.3	0.5	0.3	0.9	1.1
Methoxyl, (%)‡	0.9	0.05	2.4	0.54	0.75
Tannin (%)	0.5	0.2	0.2	0.6	0.4
Specific rotation (degrees)‡	−33	+25	+11	+23	+42
Intrinsic viscosity (ml/g)‡	8	15	37	6	20
Neutralization equiv.‡	810	1975	960	720	855
Hence uronic anhydride§	22	9	18	25	21
Sugar composition after hydrolysis (%):‖					
4-*O*-Methylglucuronic acid	5.5	0.5	14	3	4.5
Glucuronic acid	16.5	8.5	4	22	16.5
Galactose	53	49	31	68	56
Mannose	0	0	5	0	0
Arabinose	23	42	29	7	21
Rhamnose	2	<1	17	<1	2

*Corrected for moisture content.
†From Table 2.
‡Corrected for moisture and protein content.
§If all acidity arises from uronic acids.
‖If all methoxyl groups located in this acid.

Table 2. Amino acid composition (residues per 1000 amino acid residues) for five gum samples

	Atalaya hemiglauca	*Cassine aethiopica*	*Combretum paniculatum*	*Pseudocedrela kotschyi*	*Sclerocarya birrea*
% Nitrogen	0.21	0.08	0.05	0.13	0.16
Alanine	88	73	78	36	41
Arginine	25	22	15	18	12
Aspartic acid	118	98	134	58	56
Cystine	25	0	0	0	0
Glutamic acid	86	78	70	42	37
Glycine	96	114	156	37	37
Histidine	17	15	27	24	36
Hydroxyproline	98	118	109	353	348
Isoleucine	35	34	33	17	17
Leucine	48	72	52	50	56
Lysine	42	46	44	48	25
Methionine	8	12	10	4	4
Phenylalanine	36	42	37	24	27
Proline	61	72	48	54	68
Serine	77	64	72	118	136
Threonine	56	53	47	46	45
Tyrosine	26	22	23	25	15
Valine	58	65	45	46	40
Hence nitrogen conversion factor	6.47	6.49	6.31	6.75	6.77

hydroxyproline and serine) which are more commonly associated [16] with *Acacia* and other genera within Leguminosae.

Sclerocarya birrea occurs widely in the drier African savannah regions, but also extends to Ethiopia and Uganda [15]. Its sugar and amino acid compositions show similarities to those reported [14] for a sample of gum from *Sclerocarya caffra*, a closely related species [8]

Table 3. Cationic composition of the ash from gum samples (μg/g ash, 550°)

	Atalaya hemiglauca	*Cassine aethiopica*	*Combretum paniculatum*	*Pseudocedrela kotschyi*	*Sclerocarya birrea*
Calcium	189 000	182 000	247 000	248 000	139 000
Cadmium	3	0	1	8	11
Chromium	7	26	5	20	25
Cobalt	13	33	0	18	4
Copper	62	29	4	45	9
Iron	89	333	165	1390	3120
Lead	4	11	11	21	76
Magnesium	54 800	56 900	52 400	31 400	17 000
Manganese	107	600	547	125	40
Nickel	2	37	3	8	1
Potassium	67 600	23 800	40 600	27 100	18 500
Sodium	165	6940	150	270	980
Zinc	37	84	347	15	13

growing in Zimbabwe. The comments above relating to the amino acid composition of *Pseudocedrela kotschyi* apply also to *Sclerocarya birrea* gum.

These five gums are of poor quality. Because of their tannin content their use in foodstuffs in any country should be avoided, but they could serve useful technological purposes in local production areas. The gum from *Pseudocedrela kotschyi* may have an unusually simple structure; an additional supply of the gum is sought to permit a structural study to be made.

EXPERIMENTAL

Origin of gum specimens. Gum samples from *Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae) and *Pseudocedrela kotschyi* Harms (Meliaceae), both from northern Nigeria, were kindly provided by Mr J. Coppen (Overseas Development Natural Resources Institute U.K.). Gum from *Combretum paniculatum* Vent. subsp. *microphyllum* Klotsch. (Combretaceae) was sent by Mr C. H. Stirton (Pretoria) and gum from *Cassine aethiopica* Thunb. (Celestraciae) was sent by Mr Th. Müller (Curator, Botanic Garden, Salisbury, Zimbabwe). Gum from *Atalaya hemiglauca* (S. Muell.) S. Muell. ex Benth., was collected by Mr L. Ulyatt (Herbarium of Northern Territory, Alice Springs, Australia).

Analytical methods. Details of the standard analytical methods used have been given recently [4, 6].

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Specifications for gum arabic (*Acacia senegal*); analytical data for samples collected between 1904 and 1989

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The regulatory specifications for gum arabic (*Acacia senegal*) are superficial and inadequate to ensure that it is not adulterated with non-permitted gums from other botanical sources. Moreover, the existing specifications do not give the consumer the essential assurance, fundamental to food safety evaluation principles, that the nature and quality of gum arabic used in foodstuffs always conforms to that of the Test Article selected for the toxicological studies which justified the current status ('ADI not specified') of gum arabic as a permitted food additive. The availability of well-preserved gum arabic samples, collected between 1904 and 1939, has enabled invaluable data to be added to those derived from samples from the most recent crops. The resulting analytical data substantiate and greatly extend the quantitative information available previously for the chemical characterization of gum arabic for regulatory and trade purposes. The data confirm that good-quality commercial gum arabic was used previously as the Test Article. There is no evidence that the specific rotation of gum arabic has become significantly less negative in recent years.

Keywords: gum arabic, specification, criteria of identity and purity, specific rotation, cationic composition, amino acid composition

Introduction

Gum arabic (*Acacia senegal* (L.) Willd.) was re-affirmed (FDA 1974) as GRAS within the U.S.A. in 1974. Following requests (WHO 1974; EEC 1974) for positive toxicological evidence of its safety, gum arabic was awarded the status 'ADI not specified' by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 1982 (FAO 1982), provided that the gum conforms to the established specifications for its identity and purity (WHO 1982). The data upon which these decisions were based have been reviewed (Anderson 1986a). In addition, as required by the regulatory authorities, the analytical data characterizing the sample of gum arabic selected by trade sponsors as the 'Test Article' were published (Anderson *et al.* 1983) together with the corresponding data for a further 12 gum arabic samples from major producing countries. This served to demonstrate that the Test Article was a reputable, representative sample of gum arabic of fair average commercial quality, fully compliant with the specifications in existence at the start of the toxicological studies. Although other gum arabic reference samples have been characterized (Anderson *et al.* 1968, 1985), data from a wider range of samples are desirable in order that acceptable average values, or ranges of values, can be established for those analytical parameters which, conjointly, serve to distinguish

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gum arabic unambiguously from other non-permitted water-soluble gums. To this end, the acquisition of additional data was begun in 1986.

The inadequacy of the present regulatory specifications for gum arabic has become increasingly more evident (Anderson *et al.* 1983, 1985, Anderson 1985, 1986a,b, Anderson *et al.* 1986, Anderson 1988a,b, Anderson and Morrison 1989a). Simple ways in which the criteria of identity and purity could be extended to give the much greater food safety assurance now urgently required have been suggested (Anderson *et al.* 1985, 1986, Anderson 1988b); such suggestions were intended as temporary improvements rather than as constituting the overall revision of the present specifications now long overdue.

As a separate issue, there have been suggestions in recent years from a few gum traders that the severe Sahelian droughts of 1973–1974 and 1983–1985, which caused heavy losses of *Acacia senegal* trees, have led to physiological adaptations in the trees which survived, resulting in changes in some of the well-established characteristic analytical parameters for gum arabic, particularly its specific optical rotation. Whilst a search for evidence of any such changes in recent gum arabic crops was in progress, eight gum arabic samples collected in the Sudan and Nigeria between 1904 and 1939 became available for analytical evaluation from an official source to provide important additional breadth to the available sample data base. In consequence, data can now be presented for a much more extensive range of gum arabic samples than has been available previously.

Experimental

Origin of gum samples

The samples studied were natural gum arabic obtained from known, reputable sources. Some samples were obtained in natural lump form, others in kibbled form; in all cases they were reduced to a fine powder, by hand with a pestle and mortar, to minimise generation of heat, to ensure representative sampling for each analysis. Spray-dried samples were avoided, to eliminate the possibility that samples had been heat-abused or subjected to any of the forms of blending or chemical pretreatment (bleaching, sterilization, acidic or auto-degradation) which some processors incorporate into spray-drying operations.

(a) Sudanese samples. Samples S1 (1904), S2 (1905), S3 (1935) and S4 (1939) were obtained from the reference collection of the U.K. Overseas Development Authority (formerly the Tropical Products Institute). The samples, which had been stored excellently in cool, dark conditions in well-stoppered glass jars, were of good, clean, normal appearance. Sample S5, supplied for reference purposes to this Department's gum research programme by Messrs Rowntree & Co. Ltd, York, U.K., in 1960, was representative of a 25-ton consignment. Samples S6 and S7 were collected by the late Mr M. P. Vidal-Hall, Gum Research Officer to the Sudan: S6 was representative of the fourth picking of the 1960 season at Quala en Nahal; S7 was representative of the second picking of the 1962 season at Goz el Ganzara. Sample S8 was collected at Goz Ashgar in 1970 by Mr A. G. Self-el-Din, Sudanese Gum Research Officer at that time. Sample S9 was supplied in 1971 by a European gum importer. Sample S10, supplied by Messrs Rowntree-Mackintosh Ltd, York, U.K.,

in 1977 for toxicological evaluation purposes, was representative of the various gum consignments approved for use by the Quality Control Laboratory of that company over a period of several months. Sample S11 was supplied for reference purposes in 1986 by a major American user. Sample S12 was provided in 1988 by a European user and sample S13, provided by a European importer, was representative of shipments of the 1988/89 crop made in March 1989. Three samples, representative of Sudanese 1987–88 shipments to American (sample A) and European importers (samples B and C) were used only in ash determinations (table 5).

(b) Nigerian samples. As for the earliest Sudanese samples (S1–S4), well-preserved Nigerian samples N1 (1905), N2 (1931), N3 (1933) and N4 (1933) were obtained from the gum collection of the U.K. Overseas Development Authority; the gum samples were labelled as originating from Bornu Province. Sample N5 was provided for reference purposes by a U.K user in 1958, and samples N6 and N7 by a U.K. importer in 1959 and 1960, respectively. Sample N8 was supplied for use in a research project in 1961 by Messrs Rowntree Ltd, York, U.K. Sample N9, from Maiduguri, northern Nigeria, was submitted to this laboratory for evaluation in 1967 by the Tropical Products Institute, London.

(c) Other samples. In addition, the following samples from recent production years were received from European importers or users for evaluation because of their unacceptable solubility, viscosity, or poor functionality as emulsifiers, which made the true identity, value, and possible commercial use of these gum parcels uncertain:- sample 'V', described as 'West African gum arabic', was received in 1986; sample 'W', described as 'Nigerian gum arabic dark No. 1', was received in 1987; samples 'X' and 'Y', described as 'Nigerian gum arabic', were received in 1988 and 1989 respectively; sample 'Z', described as 'East African gum arabic' was received in 1989.

Analytical methods

The standard analytical methods for gum exudates of this type have been described (Anderson *et al.* 1986): the quantitative assays for tannin content and cation composition were described more recently (Anderson and Morrison 1989a).

Results

The data obtained for Sudanese gum arabic samples are shown in tables 1, 3 and 5, and for Nigerian gum arabic samples in tables 2, 4 and 6. Table 7 compares the data from this study with the ranges, means, and standard deviations for previously published data, and enables the data for the Test Article used in toxicological evaluations (Anderson *et al.* 1983) to be compared with the overall mean values ($n = 35$) for the data available. Table 8 compares the data (mean ± standard deviation) from this study ($n = 22$) with that previously published for amino acid compositions, nitrogen content and specific rotations ($n = 19$) of gum arabic samples. Table 9 gives some additional data and table 10 presents some of the data obtained for the purposes of establishing that samples 'V, W, X, Y and Z' are not acceptable commercial samples of gum arabic.

Table 1. Analytical data for Sudanese gum arabic samples.

	Sample number and year of origin													Hence	
	S1 1904	S2 1905	S3 1935	S4 1939	S5 1960	S6 1960	S7 1962	S8 1970	S9 1971	S10 1977	S11 1986	S12 1988	S13 1989	Mean	S.D.
Loss on drying, 105°C (%)	14	14	14	13	13	12	12	13	13	14	12	13	14	13	0·8
Total ash, 550°C (%)	3·8	3·9	3·2	3·7	3·7	2·8	3·6	3·4	3·4	4·8	3·5	4·1	3·6	3·6	0·4
Nitrogen (%)	0·38	0·34	0·36	0·38	0·32	0·36	0·36	0·31	0·38	0·36	0·27	0·32	0·32	0·34	0·03
Conversion factor[b]	6·72	6·57	6·66	7·04	6·54	6·44	6·47	6·71	6·66	6·31	6·70	6·70	6·57	6·62	0·2
Hence protein %[a]	2·6	2·2	2·4	2·7	2·1	2·3	2·3	2·1	2·5	2·3	1·8	2·1	2·1	2·3	0·2
Methoxyl (%)[c]	0·21	0·25	0·20	n.d.[f]	0·33	0·22	0·28	0·25	0·32	0·39	0·18	0·21	0·29	0·25	0·06
Specific rotation (degrees)[c]	−32	−31	−30	n.d.[f]	−32	−29	−31	−31	−31	−29	−28	−30	−32	−30	1·4
Intrinsic viscosity (ml/g)[c]	16	17	19	n.d.[f]	19	14	14	16	15	17	17	14	20	16	2
Brookfield visc., 25% (cp)	85	65	85	(290)[f]	90	60	70	80	60	85	80	75	100	78	13
pH, 25% aq. soln, at 25°C	4·2	4·3	4·3	4·6	4·3	4·2	4·3	4·5	4·2	4·5	4·7	4·6	4·4	4·4	0·5
Equiv. weight[c]	1020	980	980	1200	950	1160	990	1120	1130	880	1300	875	1090	1050	95
Hence uronic anhydride[d]	17	18	18	15	18	15	18	16	16	20	14	20	16	17	2
Sugar composition[c] after hydrolysis (%)															
4-*O*-Methylglucuronic acid[e]	1	1·5	1	15	2	1	2	1·5	2	2·5	1	1	2	1·5	0·5
Glucuronic acid	16	16·5	17		16	14	16	14·5	14	17·5	13	19	14	16	1·7
Galactose	37	33	32	45	48	50	46	45	48	43	42	50	47	44	6
Arabinose	29	34	34	29	23	23	20	23	23	22	30	17	21	25	3
Rhamnose	14	15	16	11	11	12	16	16	13	15	14	13	16	14	2

[a] Corrected for loss on drying. [b] From table 3. [c] Corrected for moisture and protein contents. [d] If all acidity arises from uronic acids. [e] If all methoxyl content present in this acid. [f] This sample gave 29% of an incompletely soluble gel.

Table 2. Analytical data for Nigerian gum arabic samples.

	Sample number and year of origin									Hence	
	N1 1905	N2 1931	N3 1933	N4 1933	N5 1958	N6 1959	N7 1960	N8 1961	N9 1967	Mean	S.D.
Loss on drying, 105°C (%)	15	14	14	14	13	13	13	13	12	13	1
Total ash, 550°C (%)	3·3	3·8	3·6	4·1	3·6	3·8	4·0	3·6	3·9	3·7	0·3
Nitrogen (%)[a]	0·36	0·34	0·47	0·37	0·39	0·31	0·32	0·31	0·29	0·34	0·03
Conversion factor[b]	6·90	6·80	6·64	6·75	6·52	6·51	6·63	6·56	6·59	6·65	0·2
Hence protein (%)[a]	2·5	2·3	3·1	2·5	2·5	2·0	2·1	2·0	1·9	2·3	0·4
Methoxyl (%)[c]	0·26	0·26	0·24	0·23	0·25	0·20	0·19	0·25	0·18	0·23	0·03
Specific rotation (degrees)[c]	−27	−30	−28	−29	−32	−32	−32	−29	−29	−30	2
Intrinsic viscosity (ml/g)[c]	18	15	19	22	20	17	19	18	16	18	2
Brookfield viscosity, 25% (cp)	60	60	90	75	110	75	110	100	75	84	19
pH, 25% aq. soln, at 25°C	4·2	4·5	4·1	4·3	4·2	4·3	4·3	4·3	4·2	4·3	0·03
Equiv. weight[c]	970	970	1060	920	980	1090	960	930	960	980	56
Hence uronic anhydride[d]	18	18	17	19	18	16	18	19	18	18	1
Sugar composition[c] after hydrolysis (%)											
4-*O*-Methylglucuronic acid[e]	1·5	1·5	1·5	1·5	1·5	1	1	1·5	1	1·5	0·3
Glucuronic acid	16·5	16·5	15·5	17·5	16·5	15	17	17·5	17	16·6	0·8
Galactose	36	50	40	46	51	52	51	51	45	47	6
Arabinose	32	23	29	24	19	21	22	18	22	23	4
Rhamnose	14	9	14	11	12	11	9	12	15	12	2

[a] Corrected for loss on drying.
[b] From table 4.
[c] Corrected for moisture and protein contents.
[d] If all acidity arises from uronic acids.
[e] If all methoxyl content present in this acid.

Table 3. The amino acid composition (residues per 1000 residues) of Sudanese gum arabic samples.

	Sample number and year of origin													Hence	
	S1 1904	S2 1905	S3 1935	S4 1939	S5 1960	S6 1960	S7 1962	S8 1970	S9 1971	S10 1977	S11 1986	S12 1988	S13 1989	Mean ($n = 13$)	S.D.
Percentage nitrogen	0·28	0·34	0·36	0·38	0·32	0·36	0·36	0·31	0·38	0·36	0·27	0·32	0·32	0·34	0·03
Alanine	24	29	23	29	26	31	29	25	31	28	24	30	23	27	3
Arginine	17	16	14	13	12	12	10	13	7	21	10	11	12	13	4
Aspartic acid	49	88	69	89	77	63	69	72	73	47	49	69	71	68	13
Cystine	10	12	0	0	0	0	1	1	1	3	0	0	0	2	4
Glutamic acid	28	40	34	46	39	42	52	55	49	42	31	36	63	42	10
Glycine	47	61	49	40	47	48	51	48	53	59	48	47	51	50	5
Histidine	41	47	48	24	47	42	40	44	47	59	46	40	53	44	8
Hydroxyproline	378	230	320	331	311	296	318	335	290	210	362	304	269	304	47
Isoleucine	11	16	10	17	13	13	12	10	7	13	11	15	10	12	3
Leucine	63	76	69	52	69	57	61	65	65	79	72	61	73	66	7
Lysine	22	26	23	21	26	26	22	28	26	26	24	24	31	25	3
Methionine	1	0	0	4	3	4	3	1	0	6	0	0	3	2	2
Phenylalanine	36	41	28	29	33	27	33	35	37	38	27	31	38	33	5
Proline	50	63	67	63	55	66	65	48	68	78	57	90	49	63	14
Serine	115	134	129	111	126	132	124	124	144	152	127	122	138	129	11
Threonine	60	69	67	56	66	85	63	66	69	86	65	63	71	68	9
Tyrosine	13	13	19	29	10	16	9	13	9	18	16	11	12	14	5
Valine	35	39	31	46	40	40	38	17	24	35	31	46	33	35	8
Nitrogen conversion factor	6·72	6·57	6·66	7·04	6·54	6·44	6·47	6·71	6·66	6·31	6·70	6·70	6·57	6·62	0·18

Table 4. The amino acid composition (residues per 1000 residues) of Nigerian gum arabic samples.

	Sample number and year of origin									Hence	
	N1 1905	N2 1931	N3 1933	N4 1933	N5 1958	N6 1959	N7 1960	N8 1961	N9 1967	Mean ($n = 9$)	S.D.
Percentage nitrogen	0·36	0·34	0·47	0·37	0·39	0·31	0·32	0·31	0·28	0·35	0·06
Alanine	22	19	27	19	26	26	26	30	21	24	4
Arginine	12	12	11	13	12	11	12	15	10	12	1
Aspartic acid	45	34	73	42	70	78	72	65	67	61	16
Cystine	0	0	0	0	0	2	0	1	0	0	0
Glutamic acid	32	23	37	29	37	51	69	57	43	42	15
Glycine	42	49	56	39	53	52	53	53	49	50	6
Histidine	37	42	51	46	51	52	48	49	55	48	5
Hydroxyproline	430	447	280	394	284	297	251	287	306	331	73
Isoleucine	8	15	11	17	14	11	16	15	9	13	3
Leucine	56	60	74	60	76	74	69	78	73	69	8
Lysine	19	15	26	16	31	26	29	30	24	24	6
Methionine	3	0	0	0	0	2	1	2	1	1	0
Phenylalanine	24	20	32	23	43	12	39	36	35	29	10
Proline	58	56	62	69	56	49	59	43	42	55	9
Serine	107	115	133	127	129	140	133	124	153	129	13
Threonine	55	57	72	63	66	72	75	64	79	67	8
Tyrosine	20	13	20	16	11	13	12	12	8	14	4
Valine	30	23	35	27	41	32	36	39	25	32	6
Nitrogen conversion factor	6·90	6·80	6·64	6·75	6·52	6·51	6·63	6·56	6·59	6·65	0·14

Table 5. The cationic composition[a] of the ash from Sudanese gum arabic samples (μg/g ash)

	Sample number and year of origin												Commercial samples from 1987–1988 crop[c]			Hence	
	S1 1904	S2 1905	S3 1935	S4 1939	S5 1960	S6 1960	S7 1962	S8 1970	S9 1971	S10 1977	S12 1988	S13 1989	A	B	C	Mean ($n = 15$)	S.D.
Percentage ash[b]	3·8	3·9	3·2	3·7	3·7	2·8	3·6	3·4	3·4	3·7	4·1	3·6	3·9	3·8	4·0	3·7	0·4
Aluminium	286	160	282	205	219	222	172	194	183	183	111	119	111	222	181	190	53
Calcium	270,000	201,000	248,000	267,000	246,000	238,000	306,000	268,000	232,000	280,000	328,000	238,000	206,000	271,000	228,000	256,000	34,000
Chromium	46	21	28	6	63	70	61	62	50	28	46	73	39	53	57	47	22
Copper	39	32	39	88	73	60	31	70	22	56	71	120	32	32	24	52	27
Iron	104	90	54	72	111	86	145	98	75	228	162	381	54	166	94	128	84
Lead	12	2	8	14	11	4	0	6	13	0	6	1	0	0	10	6	2
Magnesium	49,000	44,000	46,000	9,670	31,000	27,000	19,000	38,000	48,000	59,000	26,000	42,000	42,000	63,000	39,000	38,000	15,000
Manganese	73	46	135	20	91	84	27	90	87	117	36	31	113	137	414	100	95
Nickel	0	0	2	0	19	0	16	31	29	5	7	26	3	5	7	10	11
Potassium	261,000	268,000	235,000	281,000	218,000	302,000	268,000	262,000	254,000	221,000	222,000	186,000	161,000	217,000	203,000	237,000	37,000
Sodium	11,300	9,000	22,000	11,900	15,200	5,300	7,800	4,700	5,200	6,800	10,000	8,800	8,400	8,900	6,000	9,400	4,480
Zinc	24	23	25	31	17	33	19	20	21	53	31	26	9	12	17	24	10

[a] As, Cd, Co and Mo all < 1ppm for all samples.
[b] Table 1.
[c] See Anderson and Morrison 1989a.

Table 6. The cationic composition[a] of the ash from Nigerian gum samples (μg/g ash).

	Sample number and year of origin									Hence	
	N1 1905	N2 1931	N3 1933	N4 1933	N5 1958	N6 1959	N7 1960	N8 1961	N9 1967	Mean ($n = 9$)	S.D.
Percentage ash	3·3	3·8	3·6	4·1	3·6	3·8	4·0	3·6	3·9	3·7	0·2
Aluminium	184	213	318	279	368	675[c]	169	372	223	311[c]	156
Calcium	385,000	339,000	350,000	357,000	266,000	286,000	208,000	360,000	294,000	316,000	56,000
Chromium	14	0	11	3	50	50	67	50	61	34	26
Copper	35	27	50	59	50	225[d]	26	19	110	66[d]	65
Iron	70	88	99	133	75	172	139	99	117	110	33
Lead	10	7	22	20	12	13	3	11	0	11	7
Magnesium	25,000	42,000	70,000	34,000	24,000	32,000	29,000	56,000	39,000	39,000	15,000
Manganese	56	22	87	58	110	55	39	39	49	57	27
Nickel	4	0	2	0	43	28	0	35	0	12	17
Potassium	257,000	166,000	289,000	237,000	188,000	212,000	236,000	243,000	160,000	221,000	43,000
Sodium	11,100	12,800	12,300	11,900	4,500	21,000	7,000	5,100	6,100	10,200	5,200
Zinc	13	10	81	23	12	159[e]	24	21	18	40[e]	49

[a] For all samples, As, Cd, Co, and Mo all < 1 ppm.
[b] Table 2.
[c] Mean = 266 ($n = 8$) if value for N6 treated as an outlier.
[d] Mean = 47 if value for N6 treated as an outlier.
[e] Mean = 25 if value for N6 treated as an outlier (Dean and Dixon 1951).

Table 7. Comparison of data from this study with ranges, means, and standard deviations for published data; and comparison of the overall mean values ($n = 35$) with those for the Test Article used in toxicological studies.

	Data published previously				This Study						Hence Overall		
		In 1983[b] ($n = 12$)			Nigerian samples ($n = 9$)			Sudanese samples ($n = 13$)					
	In 1968[a] ($n = 1$)	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Mean ($n = 35$)	S.D.	Test Article[b]
Loss on drying (%)	n.d.	12·9/16·4[d]	14	1·1	12/15	13	1	12/14	13	0·8	13	0·9	[6][c]
Total ash (%)	3·9	3·2/5·1	4·1	0·5	3·3/4·1	3·7	0·3	2·8/4·1	3·6	0·4	3·8	0·4	[3][c]
Nitrogen (%)	0·29	0·23/0·46[d]	0·33	0·02	0·29/0·39	0·34	0·03	0·27/0·38	0·34	0·03	0·34	0·03	0·31
Methoxyl (%)	0·25	0·14/0·37[d]	0·24	0·07	0·18/0·26	0·23	0·03	0·18/0·39	0·25	0·06	0·24	0·06	0·26
Specific rotation	−30	−25/−40	−30	0·8	−27/−32	−30	1·9	−28/−32	−30	1·4	−30	1·3	−30
Intrinsic viscosity	13	13/20	17	2·3	15/22	18	2·1	14/20	16	2·1	17	2	17
Equivalent weight	1100	860/1270	1040	54	920/1090	980	56	875/1300	1050	95	1030	70	1020
Uronic anhydride (%)	16	14/20	17	2·4	16/19	18	0·94	14/20	17	1·9	17	2	17
Sugar composition after hydrolysis (%)													
4-*O*-Methyl glucuronic acid	1·5	1/3[d]	1·5	0·8	1–1·5	1·5	0·08	1/2·5	1·5	0·5	1·5	0·5	1·5
Glucuronic acid	14.5	12·5/19	16	2·3	15–17·5	16	0·8	13/19	16	1·7	16	1·5	15·5
Galactose	44	41/53	46	3·2	36/52	47	5·8	32/50	44	6	45	5	45
Arabinose	27	21/27	24	2·6	18/32	23	4·6	17/34	25	2·8	24	3	24
Rhamnose	13	10/16	13	2·1	9/15	12	2·0	11/16	14	1·9	13	2	14

[a] Anderson *et al.* 1968.

[b] Anderson *et al.* 1983.

[c] Data not comparable for these parameters.

[d] After rejection of an outlier (Dean and Dixon 1951) reported (Anderson *et al.*, 1983) for an atypical variant of *A. Senegal* in Oman which is not a source of commercial gum arabic.

Table 8. Comparisons of data (mean ± S.D.) from this study with previously published mean data for amino acid compositions, nitrogen contents, and specific rotations.

	Published 1985[a]		This study					
			Nigerian samples		Sudanese samples		Hence overall	
			1905–1933	1958–1967	1904–1939	1960–1989	Mean	
	(*n* = 8)	(*n* = 11)	(*n* = 4)	(*n* = 5)	(*n* = 4)	(*n* = 9)	(*n* = 41)	S.D.
Specific rotation (degrees)	−31 ± 1·7	−30 ± 3·7	−29 ± 1·3	−31 ± 1·6	−31 ± 1	−30 ± 1·4	−30·3	2·1
Nitrogen (%)	0·35 ± 0·11	0·28 ± 0·09	0·38 ± 0·06	0·32 ± 0·04	0·36 ± 0·02	0·33 ± 0·03	0·33	0·06
Alanine	31	28	22	25	26	27	27	4
Arginine	7	5	12	12	15	12	9	3
Aspartic acid	60	50	49	70	74	65	60	15
Cystine	1	0	0	0	5	1	1	3
Glutamic acid	36	29	30	51	37	40	37	12
Glycine	49	41	47	52	49	50	47	9
Histidine	51	44	44	51	40	46	46	7
Hydroxyproline	274	328	388	285	315	299	311	59
Isoleucine	14	12	13	13	14	11	13	3
Leucine	75	67	63	74	65	66	69	8
Lysine	26	23	19	28	23	26	24	4
Methionine	0	1	1	1	1	2	1	2
Phenylalanine	29	22	25	32	33	33	28	7
Proline	77	88	61	49	61	64	70	11
Serine	137	136	121	135	122	132	132	12
Threonine	77	76	62	71	63	70	72	8
Tyrosine	11	10	17	12	18	12	12	5
Valine	45	36	31	33	38	34	37	7
Hence Nitrogen conversion factor	6·60 ± 0·14	6·68 ± 0·24	6·75 ± 0·20	6·56 ± 0·18	6·77 ± 0·11	6·55 ± 0·14	6·64	0·18

[a] Anderson *et al.* 1985.

Table 9. Limited data for some gum arabic samples received mid-1988.

	Sudanese				Nigerian		West African	
	A	B	C	D	E	F	G	H
Specific rotation (degrees)	−31	−29	−29	−30	−29	−30	−32	−31
For 25% aq. solutions:								
Brookfield viscosity (cp)	78	88	95	80	85	480[a]	135[a]	85
pH	4·6	4·7	4·7	4·7	4·7	4·8	4·6	4·8

[a] Decreased to 100 cp after storage of the powdered gum for 3 months.

Discussion

Tables 1 and 2 show the data obtained, respectively, for thirteen Sudanese and nine Nigerian samples of gum arabic collected over a wide range of years. A comparison of the mean values and standard deviations calculated for these samples indicates that there are close similarities between Sudanese and Nigerian samples, particularly in regard to their ash, nitrogen, methoxyl and specific rotations, with the Nigerian samples tending, on average, to be very slightly more viscous and more acidic, but with slightly lower rhamnose contents.

Tables 3 and 4 show the data obtained for the amino acid compositions, and hence the nitrogen conversion factor relating nitrogen to percentage peptide/protein, for the Sudanese and Nigerian samples, respectively. For these parameters, also, there is little difference, on average, between Sudanese and Nigerian samples. Many of the individual amino acid values (residues per 1000 amino acid residues) are virtually identical; only the proline content (63 *vs.* 54) differs by more than 10%. The close similarity between Sudanese and Nigerian samples is reflected, in perhaps the most representative way, by the nitrogen conversion factors, viz. 6·62 ± 0·18 (Sudanese samples) versus 6·65 ± 0·14 (Nigerian samples).

Tables 5 and 6 show the data obtained by atomic absorption for the cationic composition of the ash derived at 550°C. In addition to the data shown, arsenic, cadmium, cobalt and molybdenum were not detected at the limit of detection 1 ppm) in any of the samples. Data published recently (Anderson and Morrison 1989a) for three Sudanese commercial samples from the 1987–1988 gum season have been added to table 5 for comparative purposes as data for S11 were not available. As a result, the mean values for 15 Sudanese samples (table 5) and nine Nigerian samples (table 6) can be compared. There are few differences in the amounts of the four major components (calcium, potassium, magnesium, and sodium). Nigerian samples tend to have higher average contents of aluminium, copper, lead and zinc; the Sudanese samples tend to have higher average contents of iron and manganese. For some commercial purposes the heavy metal content can be important; the presence of traces of copper and iron can be detrimental when gum arabic is used in emulsion polymerization systems. The undesirable presence of more than the minimum possible amounts of aluminium and manganese in foodstuffs has received comment recently (Anderson and Morrison 1989a). There are no indications, from the data now available for a period of about 80 years, of any self-consistent change, through time, in the content of any of the cations evaluated.

Table 10. Data for unacceptable commercial samples falsely claimed to be 'gum arabic'.

	Samples and sources					Values[a] for gum arabic	
	'V' West Africa, 1986	'W' Nigeria 1987	'X' Nigeria 1988	'Y' Nigeria 1989	'Z' East Africa, 1989	Mean	S.D.
Nitrogen (%)	0·23	0·24	0·19	0·37	0·26	0·34	0·03
Methoxyl (%)	0·40	0·3	0·6	0·64	n.d.	0·25	0·06
Specific rotation (degrees)	−16	−29	−4	−21	+66	−30	1·4
Intrinsic viscosity (ml/g)	30	40	12	n.d.	4	16	2·1
Brookfield viscosity, 25% (cp)	370	520	40	1460[b]	35	60–110	
Equivalent weight	1250	1150	1200	1030	n.d.	1050	95
Hence uronic anhydride	14	15	15	17	n.d.	17	1·9
Tannin (%)	1·5	1·9	0·8	n.d.	0·8	absent	
Sugar composition after hydrolysis (%)							
4-*O*-Methylglucuronic acid	2·5	2	4	4	n.d.	1·5	0·5
Glucuronic acid	5.5	8	11	13	n.d.	16	1·7
Galactose	31	27	37	62	n.d.	46	6
Arabinose	51	55	42	16	n.d.	25	3
Rhamnose	4	3	6	5	n.d.	14	2
Galacturonic acid	6	5	0	0	n.d.	absent	

[a] From table 7.
[b] This sample gave a gel rather than a viscous solution.

Table 7 presents values calculated from data published previously in 1968 ($n = 1$) and 1983 ($n = 12$), and makes comparisons with values for the ranges, means, and standard deviations calculated from the data in tables 1 and 2. There are no significant differences between the range, mean, and standard deviation for any of the parameters evaluated previously (Anderson *et al.* 1983) and those evaluated in this study (tables 1 and 2). Table 7 also shows the overall mean value calculated for each parameter from the total number of samples studied to the extent necessary so far ($n = 35$).

Comparisons, in turn, are then possible with the data (Anderson *et al.* 1983) which characterized the Test Article used in the toxicological evaluations of gum arabic between 1978 and 1982; these studies have been summarized (Anderson 1986). The Test Article selected not only complied with the regulatory specification and criteria of identity and purity in existence in 1978 but also was, moreover, representative of food-grade gum arabic originating from any of the world's major gum-producing areas. It can now be seen (table 7) that all analytical parameters for the Test Article correspond very closely with the overall mean ($n = 35$) values now available which, in effect, differ remarkably little from the mean values for $n = 12$ established in 1983. Indeed, the most divergent parameter for the Test Article is the nitrogen content (0·31%), a value which nevertheless falls within the previous (0·33 ± 0·02%) and new (0·34 ± 0·03%) limits.

Table 8 enables comparisons to be made between the amino acid compositions, nitrogen content, nitrogen conversion factor and specific rotation for samples studied previously (Anderson *et al.* 1985) and those reported in tables 3 and 4. In this case, Sudanese data ($n = 13$, table 3) are subdivided into those for 1904–1939 samples ($n = 4$) and those for 1960–1989 ($n = 9$); Nigerian data ($n = 9$, table 4) are subdivided into those for 1905–1933 ($n = 4$) and for 1958–1967 ($n = 5$). Data are presented in terms of mean ± standard deviation; calculated values for the overall means ($n = 41$) are shown. The objective was to expose indications of the nature and extent of any differences in these parameters between the earlier and more recent production years, within the limitation that data are available for only four older samples from each source. Although all mean values for each subset fall well within the limits of the overall mean ± SD, the mean nitrogen content (0·36%) for the earlier Sudanese, and for the earlier Nigerian (0·38%) samples is slightly higher (0·36%) than the overall mean (0·34%, $n = 35$, table 7; 0·33%, $n = 41$, table 8) as are the nitrogen conversion factors (6·75 ± 0·20 in comparison with 6·65 ± 0·14 for Nigerian samples (table 4); 6·77 ± 0·11 in comparison with 6·62 ± 0·18 (table 3) for Sudanese samples). The only slightly atypical amino acid contents are the lower serine and threonine contents and slightly higher tyrosine, aspartic acid and hydroxyproline content in some individual early samples: there is no general systematic trend.

There is no evidence, from the available data, that the specific rotation of gum arabic has changed to a less negative value in recent years. Some traders claim that a drastic change from −30 to approximately −20 degrees has occurred, and have suggested that this is a result of physiological adaptations by those *Acacia senegal* trees which survived the severe droughts in 1973/1974 and/or 1984/1985. Table 1 shows clearly, however, that samples from seasons later than 1977 have maintained specific rotations within the range −28 to −32 degrees, in line with the long-term mean value and range established for 35 samples (table 7). Some comments on this unsubstantiated claim may be offered. When gum arabic shortages occur, surplus

supplies of gum talha (*Acacia seyal*) with a strongly positive specific rotation (+56 degrees) flood the market and there are commercial pressures for blending operations to increase to help conserve supplies of pure gum arabic for special applications in which other hydrocolloids cannot be used satisfactorily. Addition of 10% gum talha (specific rotation +56 degrees) to 90% gum arabic (−30 degrees) lowers the specific rotation to −21 degrees; an attractive cost–benefit results because the cost of gum talha is only approximately 30% of that of gum arabic. There may also have been a short-term tendency, at the height of the gum arabic shortage in 1985, for the customary strict segregation of gum talha from gum arabic and for the normal grading standards of the latter to be temporarily suspended in the producing countries. Gum consumers with the strictest specifications have, however, been able to purchase gum arabic with a specific rotation of −27 to −33 degrees, since the resumption of adequate supplies in 1986. It would, of course, be lucrative commercially if it could be claimed that gum arabic trees had suffered a rapid and profound physiological change, with a resulting drastic change in gum arabic's key analytical parameter, in order to disguise a preference for marketing admixtures with gum talha, which is not permitted in foodstuffs and for which no toxicological evidence of safety of any kind exists.

In order to investigate the 'decreased specific rotation' suggestion as fully as possible, measurements were made on a wider range of samples than could be subjected to the lengthy complete chemical characterization deemed desirable for inclusion in tables 1–6. Table 9 shows partial data for eight gum arabic samples received from various sources in the summer of 1988: none had unusual specific rotations, but one gave an exceptionally high initial viscosity which decreased, however, to a more normal value after storage of the powdered gum for 3 months. This behaviour, attributed to freshly collected 'green gum', is well known to gum traders. Of greater interest was the observation that some gum shipments in 1986–1989 (see also table 1) gave 25% aqueous solutions having a pH value (4·6–4·8) slightly higher than usual (4·3–4·4, see tables 1 and 2). This discrepancy was initially of concern to companies which had included this simple measurement in the in-house quality assessment specifications, which they had found it necessary to establish because of the inadequacy of the current regulatory specifications to ensure that good-quality gum arabic will, beyond doubt, be supplied when it is ordered. Some companies were forced to formulate their own gum arabic specifications for such reasons in the mid-1970s. Despite their high pH value, the quality and functionality of some of the samples listed in table 9 was found to be satisfactory.

Table 10 shows partial data for five unacceptable gum samples, representative of many more received for evaluation since 1985 from gum traders and users. These samples, purporting to be gum arabic, had been offered for sale, usually by little-known shippers, under misleading miscellaneous trade descriptions at prices well below the export price fixed by the Sudanese Gum Arabic Company. Such samples can easily be demonstrated not to be authentic gum arabic, defined as originating from *Acacia senegal* (L.) Willd. or its related species: in addition to the following analytical distinctions, they failed to act as emulsifiers. Sample 'Z' was the most easily rejected; it has a highly positive specific rotation, a tannin content, and unusually low viscosity. East African gums of such type are usually derived from positive rotation *Acacias* such as *A. drepanolobium*, *A. tortilis*, *A. xanthophloea* etc., for which analytical data exist (Anderson and Dea 1968, Anderson and Bell 1974, Anderson *et al.* 1984) or from *Combretum* species (Anderson *et al.* 1986).

Samples 'V', 'W', 'X' and 'Y' were somewhat more plausible; all have negative specific rotations, with sample 'W' having the same value as true gum arabic. Many users have been deceived in this simple way in the past. It is fairly generally recognized that gums with positive specific rotations, or with negative rotations outwith the range -27 to -33 degrees, cannot be gum arabic from *Acacia senegal*. But gums from other genera, or blends of gums carefully formulated to give specific rotations within the range -27 to -33 degrees, must not automatically, *per se*, be accepted as gum arabic: there must be a close correspondence of all of the other analytical parameters as well. Thus samples 'V' and 'W' are much too viscous, have completely different ratios of galactose to arabinose, and have much too low rhamnose contents: even more characteristically, they contain tannin and also galacturonic acid, which has never been found in any authentic *Acacia* exudate and is an important characteristic of the non-permitted *Combretaceae* gums (Anderson *et al.* 1986). Had this degree of adverse evidence not been deemed to be sufficient to resolve a trade dispute, more expensive additional analyses, involving amino acid compositions and/or nuclear magnetic resonance spectroscopy, have proved to be conclusive. Sample 'X' is rejected because of its low specific rotation, nitrogen, and rhamnose content, its high methoxyl content, its tannin content and incorrect galactose/ arabinose ratio. Sample 'Y', similarly, has a high methoxyl and low rhamnose content together with an incorrect ratio of galactose to arabinose; in addition, sample 'Y' gave an exceptionally high viscosity plus a high proportion of insoluble, gel-like material and a reactivity to iron similar to that given by *Albizia* gums (Anderson and Morrison, 1989b).

These samples are typical of the poorer-grade gum parcels which are continuously on offer, and which reputable importers and end-users must exert continuous vigilance to differentiate from gum arabic, and to reject. Such parcels, frequently offered at very low prices in West and East African ports, or from Indian or South/Central American sources, usually comprise admixtures of gums from various non-permitted plant genera. This arises when gum is collected by nomads in remote areas where sparse natural, mixed, populations of a wide variety of gum-bearing trees occur. In contrast, the great majority of Sudanese gum is derived from cultivated plantations, developed over many years as monocultures of *Acacia senegal* derived from tree-improvement programmes; this gives a greater degree of confidence, relative to that for most other geographical locations, that the gum shipped originated from a single, permitted species. This is one essential prerequisite from a quality viewpoint.

Conclusions and regulatory implications

The data presented are the most extensive available for the chemical characterization of gum arabic. The samples, obtained over many years from a wide range of reputable sources, have been studied as part of the general continuing extension of knowledge of *Acacia* gum exudates and related food safety assurance considerations contributed by the Gum Research Group of this Department. The analytical studies involved were not undertaken at the specific request of any regulatory body, nor of any company or organization having vested commercial interests.

The data greatly extend, but also strongly substantiate, those available previously. Greater assurance results from the ranges, means, and standard devia-

tions that can be calculated from the data for a much greater number of samples originating over a much wider time-span.

In particular, there is no evidence to support a recent controversial suggestion that *Acacia senegal* trees have undergone surprisingly short-term evolutionary changes such as might have resulted in the marked changes in the nature, composition, and structure of gum arabic that would be necessary to cause a very sensitive indicator, the specific optical rotation, to change by 30% (i.e. from −30 to −20 degrees). To the contrary, there are convincing indications that gum arabic, as exuded by *Acacia senegal* (L.) Willd., has remained remarkably constant, not only in its specific rotation but also in other analytical parameters, over the past 80 years. As for any natural product of such great complexity, seasonal and geographical variations in composition and properties result in a valid range of values for each of the analytical parameters: the relatively small extent of these, first demonstrated 20 years ago (Anderson *et al.* 1968) has been confirmed here.

The new data also clearly substantiate the previous evidence, based (Anderson *et al.* 1983) on data for 12 samples, that the sample of gum arabic, selected with care by trade sponsors to serve acceptably as Test Article for mandatory toxicological studies in animals and in humans, was a fair, representative sample of commercial food-grade gum arabic, compliant with all the then existing regulatory chemical specifications which included a non-specified negative rotation, to which a minimal microbiological specification was added subsequently. Commercial gum arabic, as for all complex natural products of tropical origin, is subject to considerable variation in the extent of its chemical and microbiological contaminants. Consequently, gum arabic can be purchased in several different grades, e.g. 'red gum' (containing larger proportions of natural colouring compounds) and 'dust' or 'siftings', which usually give darker-coloured solutions of much higher mineral and microbiological content. For food safety, an acceptable microbiological quality of gum arabic is of critical importance; spore-forming thermophilic bacilli may be present in some shipments and there has been a recent warning (Abdalla 1988) of the possibility of the presence of aflatoxin-forming *Aspergillus flavus*, as recorded by other investigators (Bokhary *et al.* 1983, Blake *et al.* 1988).

As it was reported (Strobel *et al.* 1982) that the immunogenicity of a dark grade of gum arabic was significantly greater than for a good grade of very pale colour, and that the alcohol-extractable natural pigment in gum arabic was capable of causing a profound irritant effect (Strobel *et al.* 1986), it is important that the quality of the gum arabic sold for human consumption complies in all respects with that of the grade of gum selected as Test Article to gain the classification 'ADI not specified' for gum arabic (FAO 1982).

Such compliance is a primary principle upon which food safety assurance for the consumer is based. It is now generally recognized that commercial trading practices frequently conflict with food safety assurance interests; unfortunately, voluntary acceptance of the principles of good manufacturing practice is not always implemented by all suppliers and processors. Legislation without sanctions is useless (Anderson 1988b). Food safety assurance can only result, therefore, when the identity and quality of every food additive used corresponds with the specification established for the all-important Test Article that was arbitrarily selected. The regulatory authorities have the responsible task of assessing the safety of food additives. To implement the basic principle of safety assurance they should also, as was foreseen at the outset of food safety regulatory discussions (FAO 1956), ensure

that the specification for each additive is reviewed as necessary, in the light of scientific and other progress, to ensure that it remains effective as a standard, particularly in terms of changing commercial practices. A specific, detailed, up-to-date specification for gum arabic is also desirable for the protection of reputable gum traders; importers of products of overseas origin are deemed in law to be the manufacturers, upon whom any subsequent contraventions of food and labelling regulations devolve.

In this regard, the long-existing specification for gum arabic has not been subject to the revisions necessary to ensure compliance with the specifications which characterize the selected Test Article. It is not suggested that all of the analytical parameters presented here need to be adopted for that purpose. But considerable revision and extension of the long established, but now inadequate, specification and criteria of identity and purity for gum arabic are necessary to give the desired food safety assurance expected by the consumer. The data presented in this paper provide for regulatory purposes the mean values and reasonable ranges for the various analytical parameters that serve to characterize gum arabic of acceptable food grade quality.

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Acacia Gum Exudates from Somalia and Tanzania: the *Acacia senegal* complex

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Key Word Index—*Acacia leucospira*; *A. cheilanthifolia*; *A. senegal*; *Acacia* sp. *nov.*; gum exudates; amino acids; sugar composition; ^{13}C NMR spectroscopy; chemotaxonomy.

Abstract—Analytical data are presented for the polysaccharide and proteinaceous components of the gum exudates from three Somalian *Acacia* spp. *viz. Acacia* sp. *nov.* (Fagg & Styles 85) and *A. cheilanthifolia* Chiov. (members of the *A. senegal* complex) and *A. leucospira* Brenan. In addition, data are given for a Tanzanian specimen of gum arabic and, for comparative purposes, for a reference specimen of Sudanese gum arabic from *A. senegal* (L.) Willd. The gum from *A. leucospira* is dextrorotatory and has a low rhamnose, high uronic acid and high methoxyl content; it therefore has affinities to the sources of commercial gum talha (*A. seyal* Del. and relatives). In contrast, the gums from *A. cheilanthifolia* and *Acacia* sp. *nov.* are laevorotatory and of special interest as they provide the first opportunity to study positively identified members of the *A. senegal* complex backed by properly documented herbarium voucher specimens. Although undoubtedly closely related species, their chemical and spectroscopic data indicate that their gum exudates, and that from the Tanzanian sample, are analytically and structurally distinct from that of *A. senegal* (L.) Willd.

Introduction

Gum arabic is defined by the Joint FAO/WHO Expert Committee as "the dried, gummy exudate from tropical and sub-tropical *Acacia senegal* trees" [1]. As such, it is the most important of the natural exudate gums permitted as food additives. The toxicological evidence for the food safety of gum arabic derived from *A. senegal* (L.) Willd., as designated E414 within the EEC, has been reviewed [2]. No toxicological evidence of safety of any kind exists for the gum from any other *Acacia* species, of which over 900 were recognized [3] in 1978.

Gum arabic exports provide the second largest source of overseas hard currency earnings for the Sudan and other Sahelian countries. In addition, *A. senegal* is ecologically important. It is a source of edible beans/pods for human consumption, provides forage under near-drought conditions for browsing animals, yields timber and firewood and its roots fix atmospheric nitrogen and help minimize the erosion of light sandy soil [4].

Brenan's authoritative review [5] of the taxonomy of *A. senegal* showed that several species are closely related to, and may be confused with, *A. senegal* which belongs to a sizeable and complex group of spicate-flowered acacias with prickles occurring either in threes or singly, but not in pairs, on the branchlets. According to Brenan [5] the best account of the relationships between *A. senegal* and its allied species within the complex was given by Ross [6]. Nevertheless, the delimitation of *A. senegal* has presented constant difficulty and was not altogether clear in 1983: specimens are often difficult to assign [5]. Although even the actual number of varieties involved is not certain, Brenan [5] was in no doubt that much of the variation recognizable in the field is taxonomically significant with the main need being for carefully collected reference materials from further field studies in north-eastern Africa, especially Somalia, which is probably the centre of diversity for this complex.

Analytical data for gum exudates can provide a sensitive way of contributing chemotaxonomic evidence [7, 8]. It was therefore important to study the gum specimens collected in Somalia from trees identified as *Acacia* sp. *nov.* (Fagg & Styles 85) and *A. cheilanthifolia* Chiov. (members of the *A. senegal* complex), and *A. leucospira*

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Brenan. The opportunity was also taken to study a gum sample described as "Tanzanian gum arabic", of unspecified botanical origin, provided by the Overseas Development Natural Resources Institute, U.K.

Results

The analytical data for the physico-chemical and carbohydrate parameters are shown in Table 1, for the amino acid compositions in Table 2, and for the cationic composition of the ash in Table 3. All of the data shown are mean values obtained from at least two (usually three) replicate determinations which did not differ by more than ±2%.

Fourier-transform ^{13}C NMR spectra for well-characterized [9] reference specimen of gum arabic [*A. senegal* (L.) Willd.] and for the other laevorotatory gums studied are shown in Figs 1–4. Spectra were recorded overnight at 50.32 MHz (Bruker WH360 spectrometer) for 10% (w/v) gum solutions in D_2O at room temperature. Repetitive runs on any gum solution give reproducible spectra which show complete coincidences of all resonances and only minor differences in signal/noise ratios depending upon the precise duration of the run.

Discussion

Acacia leucospira Brenan, endemic in Somalia, is a multi-stemmed, low, flat-topped shrub with a yellow, papery bark and white, matted, woolly indumentum with long spinescent stipules, in pairs, and with capitate inflorescences [6]. These latter two features have long been recognized as indicative [3] of *Acacia* spp. of Bentham's [10] series *Gummiferae*, yielding dextrorotatory gum exudates [3]. These are not permitted in foodstuffs, but meet a commercial demand for technological applications; they are marketed commercially as "gum talha", mainly derived from *A. seyal* Del., *A. hockii* De Wild. and *A. ehrenbergiania* Hayne. The data shown in Table 1 confirm that the gum from *A. leucospira* is indeed dextrorotatory (+31°) and of the *A. seyal*-type in having a comparatively high methoxyl and negligible rhamnose content. *Acacia leucospira* gum differs from other gums within the *A. seyal* group [11], however, in having comparatively high nitrogen and uronic acid contents and high viscosity.

Acacia cheilanthifolia Chiov. is closely related to *A. senegal*, differing from it in having three or four (rarely five) pairs of leaflets per pinna and narrower pods [6]. The data in Table 1 indicate that the gum from *A. cheilanthifolia* and from *Acacia* sp. *nov.*, identified by Fagg and Styles as a member of the *A. senegal* complex, are laevorotatory, as is characteristic of *Acacia* spp. of Bentham's series *Vulgares* [10], and have some

TABLE 1. ANALYTICAL DATA FOR GUM SAMPLES

	A. leucospira Brenan	*A. cheilanthifolia* Chiov.	*A.* sp. *nov.* (F. & S. 85)	*A. senegal* ex Tanzania	*A. senegal** (L.) Willd. ex Sudan
Loss on drying (%)	13.7	13.7	13.8	12.8	13.6
Total ash, 550°C (%)	5.7	1.7	4.8	2.8	4.1
Nitrogen (%)	1.2	0.35	0.47	0.47	0.33
Nitrogen conversion factor (Table 2)	6.65	6.43	6.55	6.86	6.60
Hence, protein (%)	8.0	2.3	3.1	3.2	2.2
Methoxyl (%)	1.7	0.65	0.4	0.1	0.26
Tannin (%)	1.6	0.6	0.6	0.6	0
Specific rotation (°)	+31	−40	−8	−14	−30
Intrinsic viscosity (ml g^{-1})	25	1	37	10	17
Neutralization equiv. wt	640	1580	1110	850	1040
Hence, uronic anhydride (%)	28	11	16	21	17
Sugar composition after hydrolysis					
4-*O*-Methylglucuronic acid	10	4	2.5	0.5	1.5
Glucuronic acid	18	7	13.5	20.5	15.5
Galactose	48	45	40	56	46
Arabinose	24	30	26	16	24
Rhamnose	<1	14	18	7	13

*Data from ref. [9].

TABLE 2. AMINO ACID COMPOSITION (residues per 1000 residues) FOR GUM SAMPLES

	A. leucospira Brenan	*A. cheilanthifolia* Chiov.	*A.* sp. *nov.* (F. &. S. 85)	*A. senegal* ex Tanzania	*A. senegal** (L.) Willd. ex Sudan
N (%)	1.20	0.35	0.47	0.47	0.33
Alanine	50	46	22	46	28
Arginine	27	16	8	0	5
Aspartic acid	103	49	37	60	50
Cystine	21	0	0	0	0
Glutamic acid	55	40	30	32	29
Glycine	77	42	52	42	41
Histidine	26	47	52	41	44
Hydroxyproline	143	288	341	324	328
Isoleucine	40	16	12	15	12
Leucine	65	88	63	60	67
Lysine	32	46	24	21	23
Methionine	5	3	0	0	1
Phenylalanine	57	16	25	21	22
Proline	70	86	67	68	88
Serine	82	111	157	121	136
Threonine	51	63	80	61	76
Tyrosine	17	11	5	16	10
Valine	78	32	25	54	36
Hence, nitrogen conversion factor	6.65	6.43	6.55	6.86	6.60

*Data from ref. [12].

TABLE 3. THE CATIONIC COMPOSITION OF THE ASH FROM GUM SAMPLES ($\mu g\ g^{-1}$ ash)

	A. leucospira Brenan	*A. cheilanthifolia* Chiov.	*A.* sp. *nov.* (F. &. S. 85)	*A. senegal* ex Tanzania	*A. senegal** (L.) Willd. ex Sudan
Ash (%)	5.7	1.7	4.8	2.8	4.1
Calcium	298,000	155,000	235,000	194,000	235,370
Cadmium	0	0	0	0	0
Chromium	35	<5	<5	<5	49
Cobalt	<5	<5	<5	<5	0
Copper	115	142	56	62	29
Iron	1350	2160	72	340	105
Lead	<10	<10	<10	216	3
Magnesium	20,600	11,200	18,800	58,280	48,250
Manganese	102	44	8	30	221
Nickel	53	35	29	<5	5
Potassium	22,900	170,000	88,300	188,200	193,700
Sodium	1650	26,000	6650	4260	781
Zinc	87	164	39	94	10

*Data from ref. [13].

analytical features comparable to, but different from, those characteristic of Sudanese gum from *A. senegal* (L.) Willd. The data therefore support the assignment of the origins of these gum samples to members of the *A. senegal* complex, i.e. species very closely related to, but distinguishable taxonomically from, *A. senegal* (L.) Willd., the classical source of Sudanese commercial gum arabic.

The analytical parameters showing the greatest differences from those established for *A. senegal* gum [9] are the methoxyl content (high in *A. cheilanthifolia* gum, low in the Tanzanian gum), the intrinsic viscosity (extremely low for *A. cheilanthifolia* gum, very high for *Acacia* sp. *nov.*) and the rhamnose content (low for the Tanzanian gum). The analytical differences reported lie well outside the limits of error inherent in the

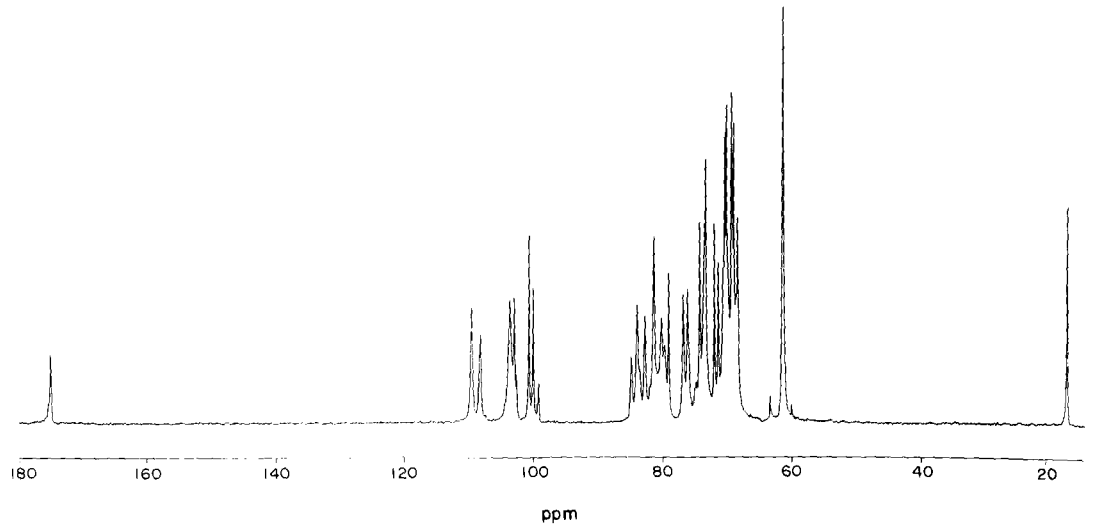

FIG. 1. FOURIER-TRANSFORM ^{13}C NMR SPECTRUM FOR GUM FROM *ACACIA SENEGAL* (L.) WILLD. (Sudanese gum arabic).

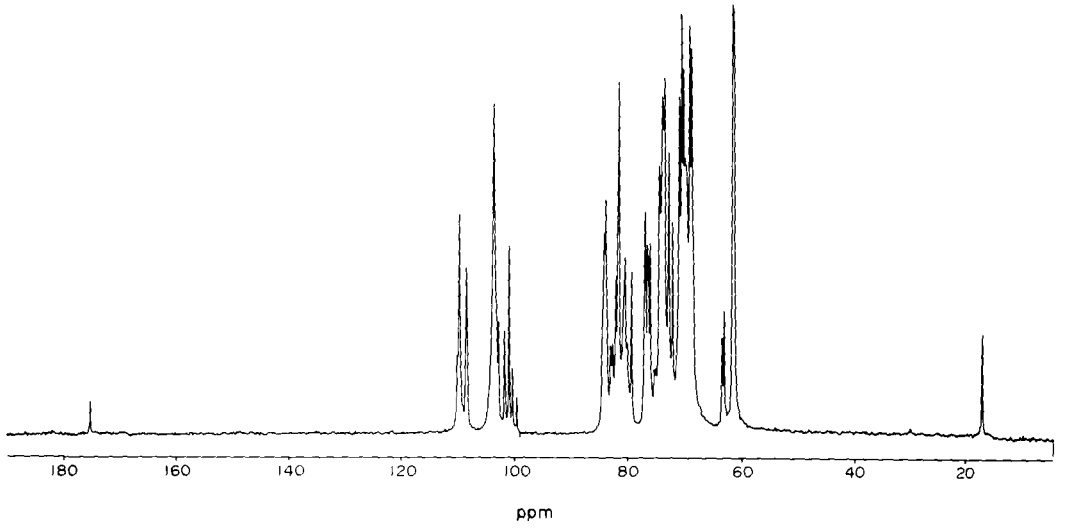

FIG. 2. FOURIER-TRANSFORM ^{13}C NMR SPECTRUM FOR *ACACIA CHEILANTHIFOLIA* CHIOV. GUM.

analytical methods used. In addition, the gums for *A. cheilanthifolia, Acacia* sp. *nov.*, and from Tanzania all contain tannin, in contravention of the EEC specification for food grade gum arabic (E414).

The amino acid data in Table 2 indicate that the peptide/protein contents of the three laevorotatory gums studied are broadly similar to that of *A. senegal* gum [12] with hydroxyproline and serine by far the most abundant amino acids present. Table 3 shows that the Somalian gums have high nickel and low magnesium contents, probably reflecting differences in the soil composition at the different collection sites. The gums from *A. leucospira* and *A. cheilanthifolia* have very high iron and copper contents, but their

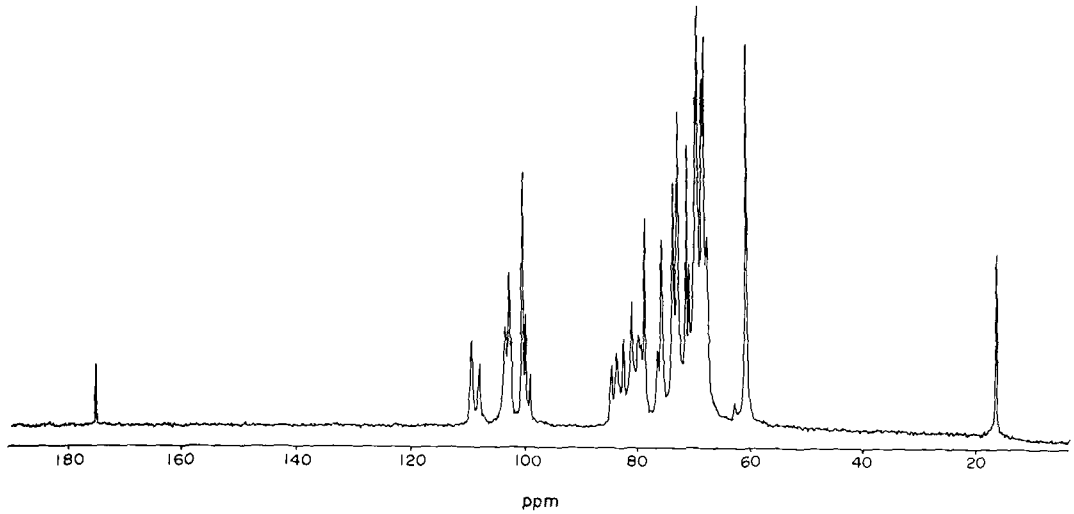

FIG. 3. FOURIER-TRANSFORM ^{13}C NMR SPECTRUM FOR *ACACIA* SP. *NOV.* (Fagg & Styles FHO 85) GUM.

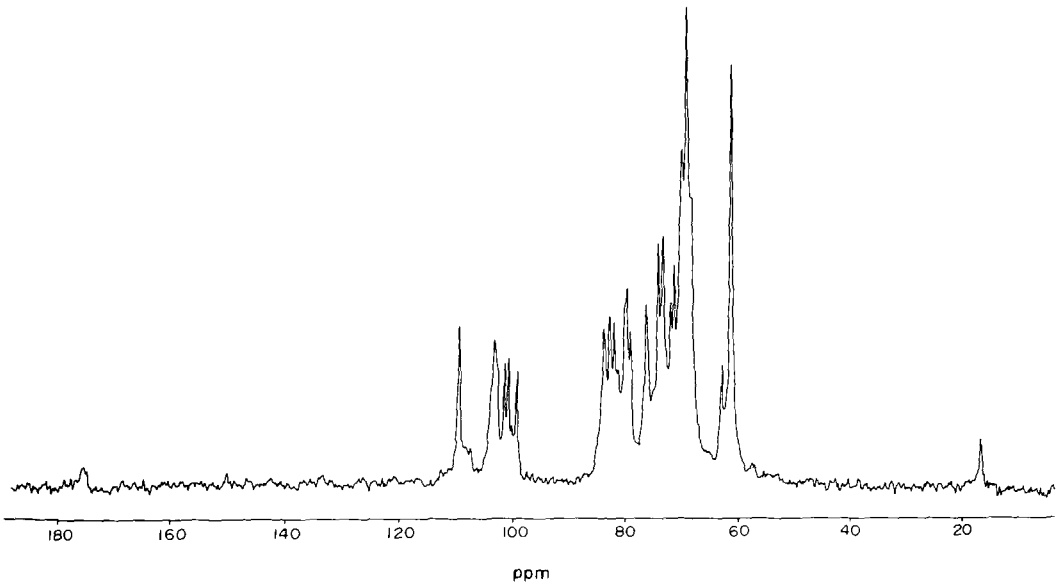

FIG. 4. FOURIER-TRANSFORM ^{13}C NMR SPECTRUM FOR TANZANIAN GUM ARABIC.

cationic compositions are not otherwise unusual [13].

Consideration of the data in Tables 1–3 leads to the conclusion that the analytical differences between the reference sample of gum from *A. senegal* (L.) Willd. and the gums from *A. cheilanthifolia, Acacia* sp. *nov.*, and from Tanzania are sufficiently extensive to indicate that they would differ in terms of their functional performance, but the very small amounts of these samples available did not allow this to be evaluated. This would be of importance in assessing the suit-

ability of these gum arabic variants for technological applications should they ever become available in commercial quantities.

Confirmation of the analytical indications that the laevorotatory samples differ is given by the fact that the ^{13}C NMR spectra (Fig. 1) for the gum for *A. senegal* (L.) Willd. and for the other gums (Figs 2–4) all differ quite extensively, although the differences are interpreted as reflecting fine, rather than major, differences in the overall structures of these extremely complex natural products. The spectrum for *A. senegal* gum (Fig. 1) confirms that first published by Artaud *et al.* [14] who also established that different samples of gum arabic from *A. senegal* gave superimposable spectra having the following assignments: *C*-methyl groups in rhamnose (16–17 ppm); $-CH_2$ groups at C6 of hexoses (60–62 ppm); —CH groups at C2–C5 of hexoses (65–85 ppm); anomeric —CH groups at C1 (95–105 ppm); C6 uronic acid groups (174–176 ppm).

It can be concluded that taxa of the *A. senegal* complex studied are closely related but that their gum exudates differ in composition and fine-structure.

Experimental

Analytical methods. The standard analytical methods for the amino acid and sugar components of gum exudates have been described [13].

Origin of gum samples. Gum samples were collected in 1987 in north-eastern Somalia by C. W. Fagg and B. T. Styles from the following three species: (a) *Acacia cheilanthifolia* Chiov. (Fagg and Styles FHO 44) in the region of Bari (9.50 N, 50.22 E), altitude 250 m. A low, rounded bush with matted stems to a height of 30 cm with a single stem rising to 4.5 m. Smooth light-grey bark; creamy white, scented flowers. Common on rocky, black soils in association with *Balanites* spp., *Commiphora* spp. and low *Acacia tortilis.* (b) *A. leucospira* Brenan (Fagg and Styles FHO 81) in the region of Muduug (6.45 N, 47.26 E), altitude 450 m. A multi-stemmed, low lying, shrub up to 20 cm tall with a crown diameter of 1–2 m. Thin, yellow, papery peeling bark; tomentose pods. Common on gypsum soils with *A. senegal, A. tortilis, Commiphora* spp. and *Boscia mimimifolia.* (c) *Acacia* sp. *nov.* (Fagg & Styles FHO 85) in the region of Golgaduud (5.45 N, 46.28 E), altitude 350 m. A single stemmed tree with twisted bole up to 5 m high, DBH 15 cm, rounded compact crown (bush form also seen). Brownish grey bark peeling to reveal yellowish papery layer underneath. Yellowish white flowers. In rocky sand in association with *Acacia* and *Commiphora* spp.

A gum sample described as "Tanzanian gum arabic" was provided by the Overseas Development Natural Resources Institute, U.K. The reference sample of gum from *A. senegal* (L.) Willd. was that used as the representative Test Article in toxicological studies [9] to establish the safety of gum arabic, as defined, as a food additive.

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confirmed spectrally by their relative proportions of the different major polysaccharide functional groups. In addition to a broad molecular weight distribution, the polysaccharide systems in gum arabic have variations in their monosaccharide composition as well as a distribution in the mode of linking and branching of certain sugar units (16,19). At least some of their spectrally distinct functional groups must be located in the more peripheral locations of the highly branched polysaccharide arrays of differing molecular mass, represented by the well-established very broad, continuous molecular weight distribution shown by gum arabic (19–22), and hence may contribute, as well as the very much less abundant but possibly more functionally efficient amino acids, to the essential lipophilic–hydrophilic balance. Such fine structural differences may contribute to the observed variations in emulsification efficiency.

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either good or bad functionality in terms of the galactose/arabinose ratios, rhamnose and nitrogen contents, and viscosity. Possibly the only tentative indication of a correlation involves the calculated nitrogen conversion factor, which appears to be lower (6.50) for the better emulsifiers and higher (6.70) for the poorer emulsifiers. Scrutiny in turn of the amino acid compositions for these samples, and for the Sudanese sample (Table III), reveals that the better emulsifiers (samples 1, 2 and Sudanese) have relatively high threonine and relatively low alanine, glutamic acid, iso-leucine, phenylalanine, tyrosine, and valine contents. The opposite is the case for the poorer emulsifiers (samples 3 and 6). These correlations also hold for three Kenyan samples (4). Ugandan sample 1 has unusually low hydroxyproline and high arginine and iso-leucine contents.

Studies of the gums from several *Acacia* species (14) have indicated that the amino acids present vary not only in their relative proportions, but also in the extent of their relative distributions between peripheral sites and the less accessible core locations within the highly branched, globular, gum macromolecules. Vandevelde and Fenyo have generally confirmed (15), and extended knowledge of, the presence in gum arabic of a fraction of high molecular mass having a high nitrogen content (16); Randall *et al.* (8) deduced that such a fraction, containing both hydrophobic protein moieties and hydrophilic carbohydrate residues, and comprising only 1–2% of the added gum arabic, is responsible for its emulsifying properties. Simple calculations show that such fractions can only be present in very small amounts, as found (8,15,16). There is excellent confirmation (17) of earlier analytical indications (15) that there are no major differences in the polysaccharide content of such fractions (8); the presence of protein in different amounts (8) in fractions does not influence the linkage pattern of the sugars (17).

The ^{13}C-NMR data (Table IV), and inspection of the selected spectra shown in Figures 1 and 2, reveal that the Ugandan samples show remarkable constancy in the locations (p.p.m.) of their major characteristic resonances, the origins of which are well-established (18). There are, nevertheless, spectral variations which reveal that fine structural differences exist between these samples, as indicated initially by the variations in analytical parameters (Table I). Thus, in comparison with the Sudanese reference sample, samples 3 and 7 have four and one additional resonances respectively. Sample 5 is deficient of two, and samples 1, 2 and 6 are each deficient of three resonances. Sample 4 shows the greatest differences, having three additional resonances but being deficient of seven. In addition, there are variations in the relative intensities of the resonances shown by each sample, particularly for the principal carbohydrate functional groups, e.g. the carbonyl resonance in the C6 uronic acid groups (175 p.p.m.); anomeric –CH groups at C1 (109.5–93.9 p.p.m.); –CH groups at C2–C5 of hexoses (84.8–68.2 p.p.m.); $-CH_2$ groups at C6 of hexoses (61.2 p.p.m.); and the C-methyl group in rhamnose (16.5 p.p.m.).

Clearly, therefore, these samples share the well-established common structural features, but some samples are rather more complex than others; there are quite considerable differences between them in terms of fine structure, as

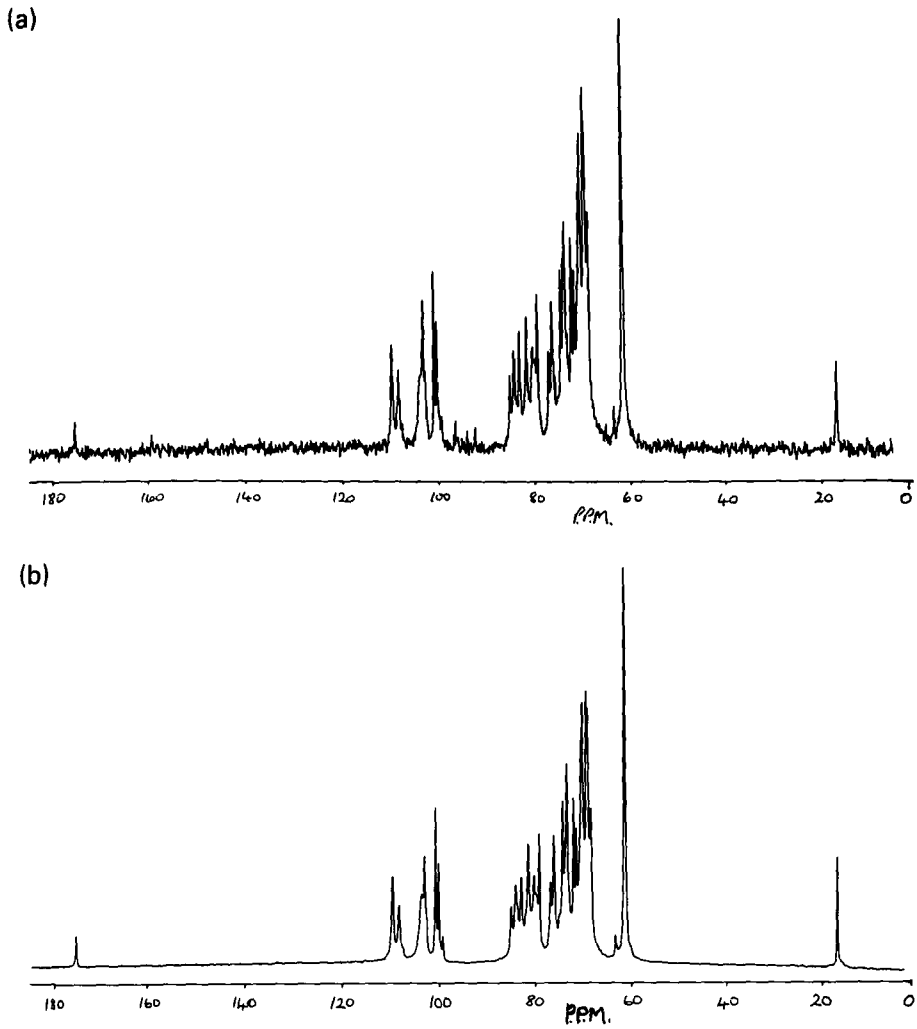

Fig. 2. ^{13}C NMR spectrum for (a) Uganda Sample 7, (b) Sudanese gum.

species. It has been confirmed (12) that the nature and distribution of the proteinaceous components of gum arabic are important (11) rather than their overall amounts. It may, moreover, be desirable (13) for a gum sample to have a mixture of both small and large protein-containing macromolecules, the former providing the emulsifying power (finer droplets initially) and the latter conferring emulsion stability (less droplet coalescence).

In comparison with the good Sudanese gum arabic sample referred to in Table I, Ugandan samples 1 and 2 are relatively efficient, and samples 3 and 6 are relatively poor, emulsifiers for limonene. Scrutiny of the analytical data (Tables I–III) fails, however, to reveal any obvious positive correlations indicative of

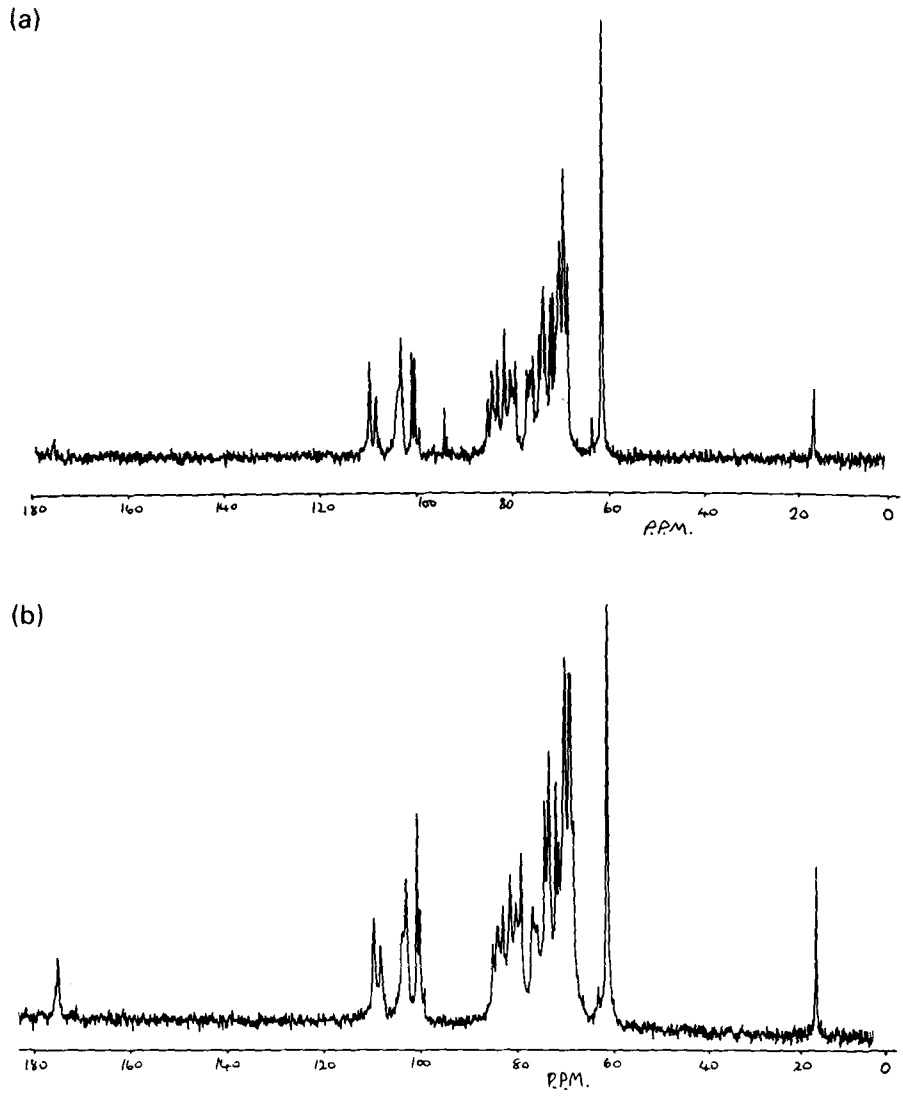

Fig. 1. ^{13}C NMR spectrum for (a) Uganda Sample 3, (b) Uganda Sample 5.

monitored carefully, and production areas possibly selected in terms of known soil types, in order that the heavy metal specifications can be met (7).

The emulsification functionality of the Ugandan samples varies widely (Table I) to an extent similar to that observed for Kenyan samples (4). The rapid mixing methods (5) of assessing emulsion functionality at single wavelength have limitations (10), but such a method has been used meaningfully (8) to assess emulsion stability, and to confirm (4) the relative emulsification efficiencies found (11) in a detailed, fundamental study of the gums from different *Acacia*

Table IV. Resonance (p.p.m.) and relative intensity data for carbon-13 Fourier-transform NMR spectra of Ugandan samples

Resonance (p.p.m.) (±0.1)	Relative intensities							
	Ugandan samples							Sudanese sample
	1	2	3	4	5	6	7	
175.0	2.42	3.23	1.38	1.19	4.22	3.38	2.00	1.71
109.5	5.03	5.13	5.55	5.00	6.51	6.52	5.78	5.35
108.1	3.30	3.36	3.58	3.60	4.87	4.74	4.52	3.56
102.9	6.90	6.46	6.94	6.60	8.83	8.60	8.04	6.65
100.6	9.04	9.68	6.13	5.23	12.67	10.99	9.50	9.90
100.0	6.07	6.20	5.77	5.51	7.03	7.17	7.04	6.19
99.1			1.84		2.40		2.03	1.57
93.9			2.99	3.43				
84.8	3.35	3.63	3.37		4.98	4.73	4.27	3.45
83.9	5.20	4.53	4.99	4.66	6.03	6.84	5.55	4.81
82.8	5.27	5.81	5.60	4.97	5.49	7.28	6.53	5.30
81.3	7.22	6.34	7.42	7.06	9.09	9.06	7.25	7.42
80.1	5.51	5.35	5.00	5.26	7.43	6.94	5.70	5.41
79.0	9.02	8.34	5.51	5.64	10.36	10.31	8.38	8.14
76.7	4.82	4.89	4.99	5.76	7.18	7.30	5.52	5.00
76.0			4.93				7.99	8.00
75.4			5.86	6.16			4.29	
74.1	10.03	9.35	7.07		13.37	12.66	9.61	10.17
73.2	12.88	11.68	9.83	9.71	16.37	13.21	11.96	12.52
72.9			7.07	8.07				
71.9	10.47	10.61	9.16	9.05	14.58	13.08	11.19	10.46
71.3	8.63	8.56	9.41	10.52	10.99	10.93	9.58	8.49
70.7			7.59					
70.2			10.5				13.90	14.15
69.9	17.73	16.06	12.31	12.51	22.64	19.78	16.46	16.53
69.1	17.48	17.45	16.47	16.66	22.64	20.75	18.66	17.19
68.9	18.25	17.30	12.41		20.95	21.14	16.98	16.05
68.2	11.53	11.08	11.05	11.76	12.21	12.95	12.47	9.79
63.2	2.77	2.43	2.27		2.51	2.79	2.76	1.65
61.2	25.11	25.11	25.11	25.11	25.11	25.11	25.11	25.11
16.5	5.47	4.99	4.02	3.44	9.64	8.56	5.04	6.63
Normalizing factor	X1.15	X1.25	X0.84	X1.14	X0.87	X1.39	X0.73	X1.00

Table III shows that, although the Ugandan samples have potassium, magnesium and sodium contents that are comparable (6) with Sudanese and Nigerian samples, they also have a considerably lower calcium content than that commonly found (6). In addition, the Ugandan samples studied have much higher levels of aluminium, chromium, copper, and iron than have been found in East African (3), Kenyan (4) and Sudanese/Nigerian (6) samples. As suggested previously (3), such variations in cationic composition, particularly for heavy metals for which upper limits are specified (7), presumably reflect the abundance of particular elements in soils at different locations. Ugandan soils are predominantly ferralites (high in iron and aluminium) or are high in copper and tin. At present these Ugandan samples are simply of academic interest as they are not available in commercial quantities. Should this position change, however, the heavy metal content of Ugandan samples will have to be

Table III. The cationic composition of the ash from Ugandan samples (μg/g ash)

	Ugandan samples							Mean[a] values for samples	
	1	2	3	4	5	6	7	ex-Sudan	ex-Nigeria
Ash (%)	3.2	3.5	3.9	4.5	3.9	3.9	4.5	3.7	3.7
Aluminium	1 940	2 210	1 270	3 380	1 470	1 000	950	190	311
Calcium	107 490	186 200	140 500	144 800	225 700	138 600	112 600	256 000	316 000
Chromium	660	1 350	710	1 560	1 170	640	870	47	34
Cobalt	12	7	<1	10	3	19	<1	<1	<1
Copper	1 020	1 740	1 270	2 830	550	455	870	52	66
Iron	1 510	1 870	830	2 490	910	730	980	128	110
Lead	23	46	35	67	26	20	18	6	11
Magnesium	34 050	35 200	35 350	40 530	52 510	38 160	23 490	38 000	39 000
Manganese	68	77	46	117	83	69	58	100	57
Nickel	18	24	13	30	12	12	6	10	12
Potassium	249 000	196 800	313 600	261 700	253 680	352 000	312 700	237 000	221 000
Sodium	8 750	1 400	4 760	9 290	10 200	12 072	1 460	9 400	10 200
Zinc	57	85	66	111	68	45	68	24	40

[a] Data from (6).

Table II. Amino acid compositions (residues per 1000 residues) for Ugandan samples

	Ugandan samples							Mean values[a] for samples	
	1	2	3	4	5	6	7	ex-Sudan	ex-Nigeria
Nitrogen (%)	0.27	0.27	0.28	0.28	0.27	0.27	0.28	0.34	0.35
Alanine	37	28	45	31	39	46	41	27	24
Arginine	19	10	14	11	15	13	13	13	12
Aspartic acid	67	49	76	56	70	72	63	68	61
Cystine	5	0	0	0	12	16	10	2	0
Glutamic acid	62	41	61	46	58	55	53	42	42
Glycine	54	40	57	47	57	58	51	50	50
Histidine	41	51	38	51	39	34	40	44	48
Hydroxyproline	179	266	216	257	232	253	240	304	331
Isoleucine	18	10	16	12	17	17	15	12	13
Leucine	72	65	67	70	69	63	66	66	69
Lysine	42	56	38	30	44	42	54	25	24
Methionine	2	0	2	2	1	2	1	2	1
Phenylalanine	44	40	53	45	48	54	49	33	29
Proline	64	70	65	82	65	49	64	63	55
Serine	113	144	109	135	105	99	110	129	129
Threonine	71	80	65	74	62	55	64	68	67
Tyrosine	16	17	27	15	20	23	25	14	14
Valine	48	33	49	36	48	49	41	35	32
Hence: nitrogen conversion factor	6.50	6.50	6.68	6.60	6.62	6.72	6.62	6.62	6.65

[a] Data from (6).

Table I. Analytical data for Ugandan samples

	Ugandan samples							Mean[a] values for samples	
	1	2	3	4	5	6	7	ex-Sudan	ex-Nigeria
Loss on drying (100°C; %)	14.2	14.8	13.9	13.2	15.2	14.7	14.2	13	13
Total ash (550°C; %)[c]	3.2	3.5	3.9	4.5	3.9	3.9	4.5	3.6	3.7
Nitrogen (Kjeldahl; %)[c]	0.27	0.27	0.28	0.28	0.27	0.27	0.28	0.34	0.35
Nitrogen conversion factor (Table II)	6.50	6.50	6.68	6.60	6.62	6.72	6.62	6.62	6.65
Hence protein (%)[c]	1.8	1.8	1.9	1.8	1.8	1.8	1.9	2.3	2.3
Methoxyl (%)[d]	0.11	0.10	0.10	0.11	0.09	0.08	0.10	0.25	0.23
Specific rotation (degrees)[c]	−32	−30	−32	−26	−34	−34	−32	−30	−30
Intrinsic viscosity (ml/g) (*in mol/dm*^{3} NaCl)[c]	15.0	14.5	12.0	13.7	16.5	15.0	19.0	16	18
Neutralization equivalent wt.[c]	1030	1060	1030	1200	1070	1180	930	1050	980
Hence uronic anhydride (%)[d]	17	17	17	15	16	15	19	17	18
Emulsion activity (limonene) (5)	1.60	1.60	1.40	1.55	1.55	1.45	1.50	1.60[b]	nd[f]
Emulsion stability (limonene) (5)	74	80	40	72	66	29	56	95	nd[f]
Sugar composition (%) after hydrolysis									
4-*O*-Methylglucuronic acid[e]	1	1	1	1	1	1	1	1.5	1.5
Glucuronic acid	16	16	16	14	15	14	18	16	16.5
Galactose	48	48	44	52	46	44	45	44	47
Arabinose	22	24	28	24	24	27	24	25	23
Rhamnose	13	11	11	9	14	14	12	14	12

[a] Data from (6).
[b] Data from (4).
[c] Corrected for loss in drying.
[d] If all acidity arises from uronic acids.
[e] If all methoxyl content located in this acid.
[f] Not done.

Results and discussion

Table I contains the analytical data for the physico-chemical and carbohydrate parameters, and also the emulsification functionality data obtained (5). Table II shows the amino acid compositions, as residues per 1000 amino acid residues. Table III shows the cation composition, expressed as micrograms per gram of the ash obtained at 550°C. Data (4,6) for Sudanese and Nigerian gum arabic (*Acacia senegal*) are included in Tables I–III for comparative purposes. Table IV records the data for the relative intensity of the characteristic NMR resonances obtained under identical instrumental operating conditions: the intensities recorded for each Ugandan sample have been multiplied by the factor (Table IV) that standardizes the intensity of their principal resonance (61.2 p.p.m.) with that (25.11) recorded initially for the Sudanese *Acacia senegal* gum sample. To facilitate visual appraisals of the nature and extent of the small structural differences represented by the variations in the spectral data (Table IV), the NMR spectra for samples 3 and 5 are shown in Figure 1, and those for sample 7 and the Sudanese sample are shown in Figure 2.

Table I shows small analytical differences between the Ugandan samples. Sample 5 has a moisture content slightly higher than that quoted (15%) in the revised specification (7), but values of 15.5% (8) and 16.4% (9) have been reported for good commercial samples. These elevated levels could easily be reduced to the specified level by storage for a short time in dry conditions, and do not reflect any food safety hazard. Likewise the total ash content of samples 4 and 7 exceeds the specified (7) value (4%) but it has also been found (6,9) that this value can be exceeded slightly by food quality samples. There is therefore a case for the regulatory authorities to be requested to raise the specified limit. The nitrogen content of the Ugandan samples fall within the range (0.27–0.39%, dry weight basis) quoted (7) in the revised specification; the values for samples 3, 4 and 7 equal that reported recently (8) for good quality Kordofan gum arabic having a specific rotation of −32.5 degrees. The methoxyl content of the Ugandan samples is low, however, in comparison with the mean values for many commercial samples (6), but their specific rotations fall within the specified limits of −26 to −34 degrees (7). One Ugandan sample is slightly more viscous (19 ml/g) and one is considerably less viscous (12 ml/g) than found (6) on average (17 ml/g) for commercial gum arabic samples. The Ugandan samples have similar uronic acid contents to typical commercial samples (6), except for sample (7) which is slightly more acidic. Table I shows that the Ugandan samples tend to have slightly lower rhamnose contents (9–14%) but have galactose/arabinose ratios that lie within the ranges recorded (6) for Sudanese and Nigerian samples.

Table II shows that there are variations in the amino acid compositions of the Ugandan samples, but these are similar in extent to those recorded (6) for Sudanese and Nigerian samples. Nevertheless, the Ugandan samples have lower hydroxyproline contents, and tend to have higher proportions of alanine, lysine, phenylalanine, tyrosine, and valine, i.e. of some of the amino acids with lipophilic rather than hydrophilic properties.

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The characterization of gum arabic (*Acacia senegal*) samples from Uganda

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Abstract. Seven samples of gum from *Acacia senegal* trees growing in north-east Uganda have been characterized. All have negative specific rotations (−26 to −34°) and nitrogen contents of 0.27–0.28% and therefore satisfy the revised JECFA specification for gum arabic. They have, however, lower methoxyl, rhamnose, and hydroxyproline contents than typical Sudanese commercial samples. In addition, the Ugandan samples vary considerably in their emulsification functionality and contain unusually high proportions of aluminium, chromium, copper and iron. Fourier-transform ^{13}C-NMR spectra confirm that the variations in the analytical parameters of these samples reflect differences in fine structure.

Introduction

Coffee and cotton are Uganda's major exports; other sources of hard currency are sought. The Steppes and *Acacia* woodlands on the sandy loams of north-east Uganda, bordering Southern Sudan, represent Uganda's driest regions. Although the occurrence of *Acacia senegal* var. *senegal* (1) and of var. *rostrata* and var. *kerensis* (2) in Uganda has been recorded, there does not appear to have been any evaluation of gum arabic of Ugandan origin.

Materials and methods

Seven small (~20 g) natural exudation gum samples were collected by the District Forest Officer of Karamoja Region in collaboration with Mr Kityo, Ugandan Ministry of Environmental Protection, in an ecological project funded by IDRC, Ottawa. Each sample comprised clean, pale-coloured gum of excellent solubility.

Analytical methods

The standard methods and instrumentation used to determine ^{13}C NMR spectra have been described (3,4). A revised version of James and Patel's method (5) was used to assess emulsification functionality. Two millilitres of a 5.0% (w/w) gum arabic solution plus 0.5 ml limonene was treated with an Ultra-Turrax homogenizer at 15 000 r.p.m. (speed 7, pre-set) for 60.0 s. The absorption at 500 nm given by a 1-in-100 dilution of the resulting emulsion is quoted as 'emulsion activity' (EA); the absorbance at 500 nm given by a subsequent 1-in-100 dilution of the lower half of that emulsion after storage for 30 min, expressed as a percentage of EA, is quoted as 'emulsion stability' (ES). A reproducibility of ~3% has been quoted (4) for such empirical assessments.

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Gum Arabic (*Acacia senegal*) from Niger—Comparison with Other Sources and Potential Agroforestry Development

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Key Word Index—*Acacia senegal*; gum arabic; revised specifications; ^{13}C NMR spectroscopy; avoidable contaminants; *Combretum nigricans*; *Acacia sieberana*.

Abstract—Analytical data and ^{13}C NMR spectra are presented for samples of gum from three *Acacia senegal* (L.) Willd. trees growing in Niger. The data show that the samples comply with the Revised (1990) Specification for gum arabic; the spectra reveal that the gums have, moreover, close structural similarities to Sudanese gum arabic. There is great scope in Niger to increase agroforestry activity based on expansion of its natural *Acacia senegal* population, but increased care will be necessary at the harvesting and marketing stages to ensure that gum from *Acacia senegal* is kept free from admixture with that from *Combretum nigricans*, *Acacia seyal* and *Acacia sieberana*, which are not permitted food additives, and which occur more extensively at present in Niger.

Introduction

Gum arabic (*Acacia senegal* (L.) Willd.) remains the most important of the natural exudate gums despite the fact that world demand has fallen from *ca* 70,000 tons p.a. in the early seventies to *ca* 24,000 tons p.a. in 1989/90. Hard currency earnings nevertheless remain substantial at the present Sudanese controlled price (2,300 U.S. dollars/tonne ex Port Sudan). The Sudan has consistently claimed to produce 85% of the world supply; this near-monopoly has disadvantages. Increased market confidence would ensue if reliable production of good quality gum arabic were to be increased in more diverse locations within the Sahel. In addition to such commercial considerations, *Acacia senegal is* ecologically important, providing highly nutritious pods/beans for human consumption, forage for browsing animals and timber/firewood. In addition, its roots fix atmospheric nitrogen and help to control the erosion of desert soils [1].

Gum arabic was accepted as a permitted food additive in 1982 [2] with the highest possible safety classification *viz.* "ADI not specified"; the toxicological evidence for safety has been reviewed [3]. Following complaints in 1986 that the existing Specification [4] was too lax to prevent gums from other than the specified source being sold and used, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently published a Revised Specification [5]. This differs in only three respects: (a) the source of gum arabic is now defined as being from "*Acacia senegal* and closely related species" (the difference involving the word "closely"); (b) the nitrogen content must be within the range 0.27–0.39%; (c) the specific rotation must lie within the range −26 to −34 degrees.

Authenticated gum samples from Niger have been difficult to obtain in the past. The opportunity to study recently acquired gum samples from *Acacia senegal* trees has therefore been welcomed.

Materials and Methods

Origin of gum samples. Gum arabic samples, from separate *Acacia senegal* trees, were collected in December 1990 at Sadoré, Niger. Gum samples were also obtained from *Combretum nigricans*, *Acacia seyal* and *Acacia sieberana* within the same locality.

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Analytical methods. Conformity with the Revised Specification [5] requires testing for solubility, acidity, precipitation by alcohol, absence of starch/dextrins and tannin; and determinations of loss on drying, total ash, acid-insoluble ash, acid-insoluble matter, arsenic, lead, heavy metals, nitrogen and specific rotation. The official analytical methods described [6] were used. Table 1 contains the data for desirable additional parameters obtained by standard methods described recently [7].

^{13}C NMR spectroscopy. Fourier-transform ^{13}C NMR spectra for 10% gum solutions in D_2O at room temperature were recorded overnight at 50.320 MHz with a Brüker WP200SY spectrometer. All spectra were recorded under identical operating conditions. To facilitate their direct comparison, the relative intensities recorded by the spectrometer for the final sample concentrations, which differed slightly through variable filtration losses, etc. were multiplied by the conversion factors shown in Table 2 to make the intensity of the principal resonance (61.2 ppm) equal for all samples.

Results

The analytical data for the physico-chemical and carbohydrate parameters of the gum arabic samples are shown in Table 1. Corresponding data for the gums from *Combretum nigricans* [8], and for gum arabic from Sudan [9], Nigeria [9], Uganda [10] and Marsabit (Kenya) [11], are included for comparative purposes. The ^{13}C NMR data for the gum arabic samples from Niger are shown in Table 2, together with that for the gum arabic Test Article [12], and samples from Uganda [10] and Marsabit, Kenya [11]. The NMR spectra for Niger gum arabic samples (a) and (b) are shown in Fig. 1. Figure 2 permits a visual comparison of the spectrum for Niger gum arabic sample (c) with that for the sample of gum arabic used as the toxicological Test Article [12].

Discussion

Gum arabic (*Acacia senegal*) remains an important international commodity despite falling demand over the past 15 years as a result of drought-induced severe shortages in 1973/74 and 1984/85, and increased availability of a wide range of extremely price-competitive modified starches. The Sudan continues to enjoy the revenue from a very large proportion of the total world tonnage sold, largely resulting from the high quality of its gum arabic. In commercial terms, quality does not simply mean clean gum of good colour, complete water solubility and low microbiological counts. Quality also implies good and reproducible functionality from a single botanical source. This arises from the fact that almost all of the Sudanese production is obtained from the agroforestry developments which, over the past 20 years, have been based on the

TABLE 1. ANALYTICAL DATA FOR GUM SAMPLES

	Acacia senegal from Niger			Revised JECFA	*Acacia senegal* from				
	(a)	(b)	(c)	Spec. [5]	Sudan (n = 13)	Nigeria (n = 9)	Uganda (n = 7)	Marsabit Kenya [9]	*Combretum nigricans* [6]
Loss on drying (%)	12.3	12.4	12.4	<15	13	13	14	13	10.6
Total ash, 550°C (%)	2.9	2.3	2.6	<4	3.6	3.7	3.9	4.6	3.0
Nitrogen (%)	0.28	0.37	0.27	0.27–0.39	0.34	0.34	0.27	0.70	0.35
Methoxyl (%)	0.17	0.24	0.15		0.25	0.23	0.10	0.10	0.24
Acetyl (%)	0	0	0		0	0	0	0	2.5
Tannin (%)	0	0	0	0	0	0	0	0	0.9
Specific rotation (degrees)	−32	−34	−34	−26 to −34	−30	−30	−31	−32	−43
Intrinsic viscosity (ml g^{-1})	19.6	19.4	15.5		16	18	15	26	35
Neutralization equiv. wt	1200	1130	1110		1050	980	1070	800	1244
Hence, uronic anhydride (%)	15	16	16		17	18	17	22	14
Sugar composition after hydrolysis									
4-*O*-Methylglucuronic acid	1	1	1		1.5	1.5	1	1	1
Glucuronic acid	14	15	15		16	16.5	16	21	8
Galacturonic acid	0	0	0		0	0	0	0	5
Galactose	44	42	45		44	47	47	54	30
Arabinose	26	27	25		25	23	25	16	41
Rhamnose	15	15	14		14	12	12	8	15

TABLE 2. RESONANCE (PPM) AND RELATIVE INTENSITY FOR ^{13}C FOURIER-TRANSFORM NMR SPECTRA OF GUM ARABIC

Resonance (ppm) (±0.1)	Relative intensities of Niger samples (a)	(b)	(c)	Mean	S.D.	Sample from Marsabit, Kenya	Gum arabic Test Article	Ugandan samples ($n = 7$)
175.0	1.69	2.12	1.75	1.85	0.23	4.80	1.79	2.54
109.5	5.42	6.07	6.11	5.87	0.39	5.40	5.10	5.65
108.1	3.30	4.00	3.72	3.67	0.35	3.72	3.24	4.00
102.9	6.31	7.87	7.11	7.09	0.78	9.84	6.40	7.48
100.6	9.19	10.29	10.10	9.86	0.59	14.48	9.13	9.03
99.1	2.85	1.68	2.28	2.27	0.58	4.56	2.27	2.09
84.8	3.54	3.67	3.33	3.51	0.17	5.52	3.42	4.05
83.9	4.80	5.88	5.77	5.48	0.59	6.24	4.97	5.40
82.8	6.39	5.71	5.30	5.80	0.55	8.04	5.63	5.85
81.3	6.39	9.18	8.32	7.96	1.43	6.24	7.13	7.63
80.1	6.02	5.75	5.74	5.84	0.16	6.12	5.51	5.88
79.0	8.50	9.40	8.61	8.84	0.49	12.72	8.82	8.22
76.7	4.96	6.35	6.36	5.89	0.80	6.24	5.00	5.78
76.0	7.92	9.02	8.46	8.47	0.55	12.36	8.19	6.46
74.1	10.07	12.04	11.19	11.10	0.99	15.24	10.35	10.35
73.2	12.27	14.07	13.62	13.32	0.94	17.76	13.51	12.23
71.9	10.65	12.13	11.14	11.31	0.75	17.04	10.80	11.16
71.3	9.77	8.38	8.45	8.87	0.78	11.64	9.13	9.80
70.2	14.38	16.15	14.83	15.12	0.92	20.52	14.83	11.97
69.9	16.44	18.10	17.09	17.21	0.86	24.36	17.39	16.78
69.1	19.56	17.60	17.19	18.12	1.26	23.52	18.80	18.59
68.9	17.85	17.62	16.60	17.36	0.66	23.40	17.52	17.84
68.2	11.85	10.77	10.51	11.04	0.71	12.72	11.18	11.86
63.2	2.40	2.32	1.72	2.15	0.37	—	2.14	2.59
61.2	25.11	25.11	25.11	25.11		25.11	25.11	25.11
16.5	9.38	10.34	10.38	10.03	0.57	11.40	9.76	5.88
Factor	×0.97	×0.97	×0.86			×1.20	×1.0	×1.0

exclusive regeneration of *Acacia senegal* within its recognized gum belt. There is therefore a very high degree of assurance that Sudanese gum arabic (as distinct from the very much cheaper gum talha) arises from one defined source. This is now of particular importance as a result of the introduction in 1990 of the considerably tighter specification [5]. It is, nevertheless, important for trade stability reasons for attempts to be made to increase production of gum arabic, capable of meeting the Revised Specification, in suitable locations outwith the Sudan.

The data in Table 1 show that the samples of gum arabic from Niger comply with the requirements of the Revised Specification [5] in terms of its four critical, quantitative requirements, *viz.* loss of weight on drying at 105°C, total ash content, nitrogen content and specific rotation; the samples also met all other requirements of the Revised Specification, described in Materials and Methods above. Table 1 also shows that the gum arabic samples from Niger correspond closely to the mean values [9] for gum arabic from Sudan and Nigeria and (with the exception of the methoxyl content) to samples from Uganda [10]. A sample from Marsabit [11] differs greatly, however.

The NMR data in Table 2 show, additionally, that the gum arabic samples from Niger are closely similar structurally, and hence in molecular properties, to a very representative sample [12] of bulk commercial gum arabic selected by sponsors for toxicological evaluation in 1980–1982. The remarkable identities of molecular structure are evident in Figs 1 and 2. The resonances at 16.5 ppm (attributable to rhamnose groups) and at 175.0 ppm (glucuronic acid groups) are sensitive indicators, as is the ratio of the relative intensities at 109.5 ppm and 108.1 ppm, which gives the relative proportions of internal/terminal arabinose groups. This approximates to 3/2 in gum arabic: the average value for the three Niger samples is 5.87/3.67, i.e. 1.60/1: that for the samples used as Test Article is 5.10/3.24, i.e. 1.57/1. It has long been known [13] that small, quantifiable

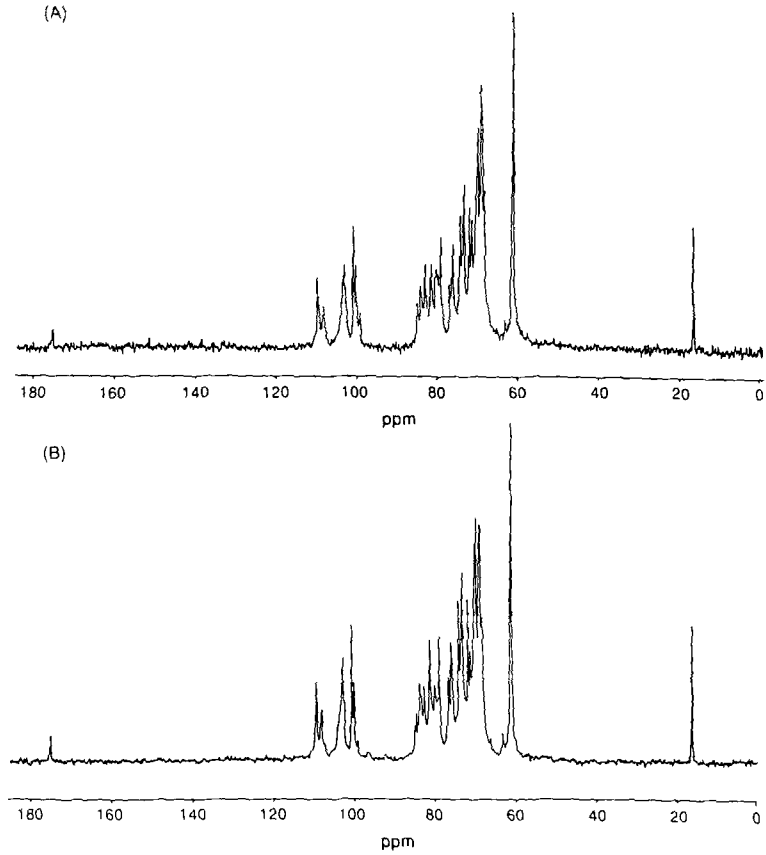

FIG. 1. ^{13}C NMR SPECTRA FOR (A) NIGER SAMPLE (a), (B) NIGER SAMPLE (b) (SEE TABLE 2).

variations in chemical composition occur between different nodules of gum arabic taken from a single tree. Data indicating the extent of these variations, and mean values for the analytical parameters for 35 Nigerian and Sudanese gum arabic samples representative of different production years between 1904 and 1989, have recently been published [9]. The NMR data in Table 2 also show, however, that the gum arabic samples from Uganda [10] differ spectroscopically in terms of the NMR resonances at 175.0, 71.2 and 16.5 ppm, with the sample from Marsabit [11] showing much more extensive differences. *Acacia senegal* is acknowledged to be a very variable species [14, 15] with recognized subspecies and varieties. The increasing acquisition of NMR spectra is now revealing that the gum arabic from trees identified as *Acacia senegal* in various parts of the Sahel may be either closely similar to (e.g. from Niger) or distinctly different from (e.g. from Marsabit) the gum from Sudan which dominate the commercial market. Whether this results (a) simply from climatological or soil differences, or (b) from more extensive hybridizations/adaptations in the *Acacia senegal* trees themselves, or from (a) and (b), may be difficult to establish in the future because of the need to control the identifiable variables sufficiently closely in any such experiments.

Climatological conditions vary considerably within Niger, with some intensely hot, arid areas that are most suitable for commercial gum production in high yield. The proven presence of indigenous *Acacia senegal* adapted to the precise ecological conditions and capable of yielding good quality gum arabic, comparable in composition

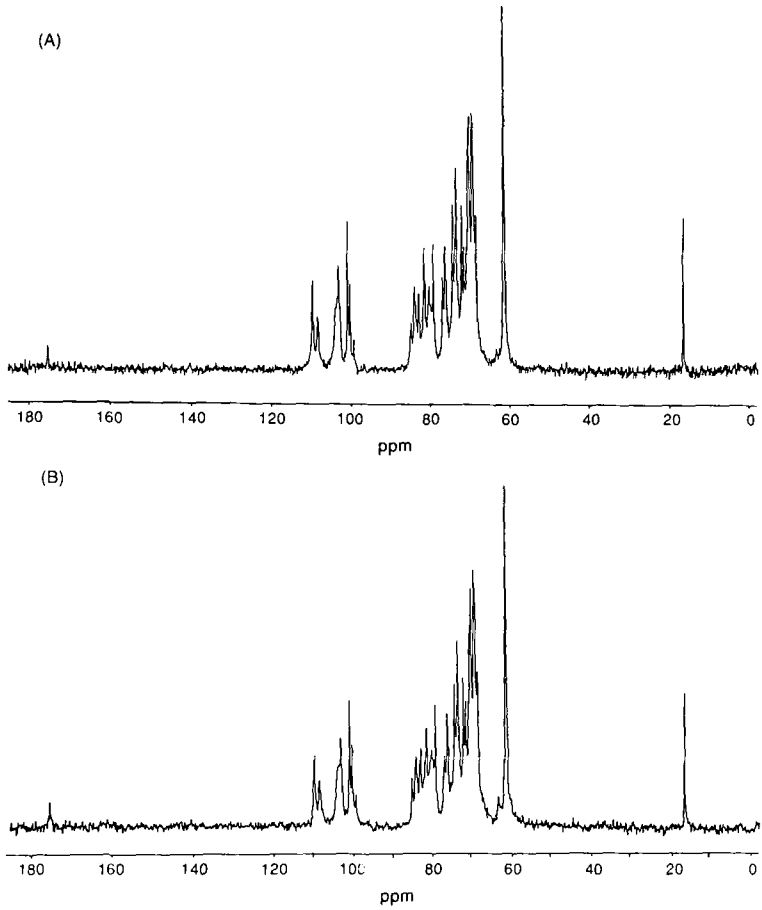

FIG. 2. ^{13}C NMR SPECTRA FOR (A) NIGER SAMPLE (c),(B) GUM ARABIC TEST ARTICLE.

and structure to the Sudanese product, offers an opportunity for attention to be given to increasing *Acacia senegal* agroforestry within Niger. It is important to emphasize that the tighter new international specifications, with concurrent tighter food safety regulations in Europe, imply that future demand must be for very high quality gum arabic derived for all commercial practical purposes from *Acacia senegal* as its closely related species, i.e. members of the *Acacia senegal* complex, are not cultivated, are relatively rare and tend to occur in locations quite distinct from the main gum production areas. There is little latitude for contamination with gum from other botanical sources such as *Combretum nigricans* Lepr. ex Guill. et Perr. [8], *Acacia seyal* Del. [16], and *Acacia sieberana* DC. [16] which occur extensively in Niger. The established analytical parameters for these gums are sufficiently distinctive to permit their detection, although this can now also be achieved much more powerfully and conclusively by NMR spectroscopy. Now that gum importers have tighter regulation specifications to meet, and are deemed to be the manufacturers of the gums they import, tighter commercial control of gum collection, cleaning/grading and marketing are necessary within Niger if the top market prices available for regular supplies of food-grade gum arabic are to be secured. Modern gum trading is based on minimum lots of 20 tonnes (container loads). The penalty for not attending to such detail is great, because the

non-food grade gums of the very plentiful, well-known, gum combretum and gum talha types only command prices of 500–750 dollars/tonne.

The ecological advantages of developing agroforestry based on *Acacia senegal* are attractive. There seems little doubt that increased production of gum arabic from *Acacia senegal*, as represented by the samples studied here, with simultaneous increased marketing attention to ensure that it does not become admixed with the non-permitted gums described, deserves consideration by Niger's research/development organizations.

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COMBRETUM NIGRICANS GUM – ITS UNUSUAL STRUCTURE/ PROPERTIES AND DIFFERENCES FROM GUM ARABIC (*ACACIA SENEGAL*)

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SUMMARY

Combretum nigricans is the major source of commercial "gum combretum" which is widely available in West African markets. Although not permitted in foodstuffs by regulatory authorities it has been used as an adulterant of gum arabic (*Acacia senegal*) with adverse results because the desirable properties of the latter are thereby greatly reduced. Gum combretum itself has an unfortunate reputation for self-adhesion to form "blocked" gum, although it is desirable for some commercial applications if stored and used appropriately. ^{13}C Nuclear magnetic resonance spectrometry and degradative procedures have shown the essential difference in chemical structure involved; the constituent rhamnose and uronic acid units, which occupy peripheral, chain-terminal structural positions in gum arabic, are located in internal positions in gum combretum.

INTRODUCTION

Combretum nigricans Lepr. ex Guill. et Perr. occurs widely as a typically variable aggregate throughout tropical West Africa, particularly in northern Nigeria, Mali and Niger. It exudes gum copiously, and contributes substantially to the tonnages available in these countries. Gum combretum varies greatly in appearance, ranging from large, dark brown or black glossy masses, through smaller, circular or oval-shaped reddish-brown lumps, to even smaller pieces that are often kidney-shaped and very pale yellow in colour. Usually one end of the nodules is strongly pigmented with an intense carmine-red substance at the point where the gum was attached to the tree bark. Poorer grades in addition do not dissolve

completely but form variable proportions of gel; regardless of the pale colour of some gum nodules, combretum gum solutions are invariably dark reddish-brown in colour. Combretum gum pieces are always smooth and opaque in appearance; this is characteristically different from the appearance of good quality gum arabic which is more crystalline in external appearance with prominent, characteristic, rough surface markings. Gum combretum also usually has a distinct acetous odour, arising from its acetyl content; this, and the colour/appearance described usually suffices for the trained eye of experienced gum buyers to differentiate gum combretum from gum arabic or to identify the extent to which gum arabic has been adulterated. This is particularly important, because of financial considerations: gum arabic (*Acacia senegal*) commands a current African selling price of 2,300 US dollars per tonne in contrast to only 600 US dollars for gum combretum. Such a price differential leads to marketing attempts to upgrade gum combretum by offering it for sale as "Dark Nigerian gum arabic No. 2" or other similarly meaningless trade terms, although it is more usually offered for sale as "Mali gum" or "gum Niger". Production of the best quality gum combretum, arising almost completely from *Combretum nigricans*, although several other *Combretum* species are of common occurrence (Anderson and Bell, 1977), appears to be centred around Sokoto in northern Nigeria.

A major disadvantage with gum combretum is its marked tendency to "block" in transit or in storage; the separate gum pieces filled into a jute sack convert into a solid mass which has to be broken up by pick-axe or sledge-hammer. This undesirable commercial occurrence is most likely to arise if the gum is delayed in transit or held in store in dockside sheds at Lagos or other West African ports after the onset of the rainy season, or stored badly during transit by sea. This is very different from the behaviour of gum arabic, which is not nearly so hygroscopic; clearly there must be a major chemical difference involved. As this has not been clearly established previously, the present study was initiated as an extension of a programme (Anderson et al, 1986; Anderson and Morrison, 1990) of fundamental studies of Combretum gums intended primarily to generate the data necessary to detect and identify Combretum gum species when used to adulterate gum arabic sold for food use. The bark of *Combretum* species is a rich source of tannins, quinonoid colouring pigments, phytotoxic and pharmacologically active

substances (Pettit et al, 1988; Singh, 1989). There is no toxicological evidence of safety of Combretum gum and it is therefore not included in the permitted lists of any of the international regulatory authorities. Detection of such adulteration of gum arabic is important for two additional reasons: Combretum gum (a) imparts an astringent, bitter taste to gum arabic, which makes adulterated consignments unfit for use in confectionery manufacture, and (b) greatly reduces the ability of gum arabic to function as an emulsifier, which makes it unacceptable for use in the production of citrus oil emulsion concentrates for soft-drink production.

By establishing the underlying reason for these various undesirable characteristics, this report is intended to assist improvement of the marketing and use of West African gum Combretum, which, by reason of its greater hygroscopicity, is much more efficient and cost-effective than gum arabic in certain technological, non-food sector applications, e.g. lithographic applications.

MATERIAL AND ANALYTICAL METHODS

Origins of gum samples

Gum collected from *Combretum nigricans* at Sadoré, Republic of Niger, in December 1990 and samples of commercial gum combretum from Mali and Sokoto, Nigeria, were used.

Analytical methods

The standard analytical methods for Combretum gum exudates have been described recently (Anderson and Morrison, 1990). Fourier-transform ^{13}C-N.M.R. spectra for 10% gum solutions in D_2O at room temperature were recorded overnight at 50.320 MHz with a Brüker WP200SY spectrometer. All spectra were recorded under identical operating conditions to facilitate their direct comparison.

Identification of uronic acids

Following complete hydrolysis of *C. nigricans* gum with 0.5 M sulphuric acid for 8 hours at 100°C, the neutral and acidic sugars were separated on a Duolite A-4 column with 5% formic acid. The acidic fraction was concentrated to a syrup. Paper chromatography on Whatman 3MM paper with solvent system A [ethyl acetate, acetic acid, formic acid, water (18:3:1:4, v/v;] gave three major

aldobiuronic acids. These were purified and shown chromatographically and by mass spectrometry to be 6-0-(β-D-glucopyranosyluronic acid)-D-galactose, 4-0-(α-D-glucopyranosyluronic acid)-D-galactose, and 2-0-(α-D-galactopyranosyluronic acid)-L-rhamnose.

Mild acidic degradation of C. nigricans *gum*

The gum was hydrolysed in 0.005 M sulphuric acid for 96 hours at 100°C. After 54 hours the intrinsic viscosity had decreased from 54 ml/g to 5 ml/g and the supernatant contained major amounts of arabinose with smaller amounts of galactose, rhamnose and di-arabinose (separation in solvent A). After neutralisation, exhaustive dialysis, and reduction to small volume, the degraded gum was isolated (yield 30%, polysaccharide A).

Smith-degradation of C. nigricans *gum*

Following small-scale preliminary experiments to establish the required conditions, *C. nigricans* gum was oxidised with 0.125 M sodium metaperiodate for 96 hours in darkness at room temperature. Excess periodate was reduced with ethylene glycol and, after dialysis for 2 days, sodium borohydride was added. After 30 hours, the resulting polyalcohol was hydrolysed. Following neutralisation, filtration and dialysis stages, the Smith-degradation product was isolated by freeze-drying (yield 35%, polysaccharide I).

Methylation analysis of C. nigricans gum, polysaccharides A and I

The classical methylation methods were used as described previously for *Combretum hartmannianum* gum (Anderson and Bell, 1976).

RESULTS

Table 1 shows the analytical data for *Combretum nigricans* gum and its degradation products, polysaccharides A and I. Table 2 gives the results of the methylation analyses. Figure 1 shows the ^{13}C-NMR spectrum for *C. nigricans* gum and its degraded polysaccharide A. Comparable data from earlier studies of gum arabic (*Acacia senegal*) are included in Table 1.

TABLE 1
Analytical data for *Combretum nigricans* gum and its degradation products: and comparable data for gum arabic*.

	Combretum nigricans Gum	Degraded Polysaccharide A	Degraded Polysaccharide I	Gum* Arabic	Degraded* Polysaccharide A	Degraded* Polysaccharide I
Nitrogen (%)	0.10	0	0.08	0.33	0	0.56
Methoxyl (%)	0.32	0.40	0	0.23	0	0
Specific rotation (degrees)	−42	+8	−26	−31	−11	−28
Intrinsic viscosity (ml/g)	54	5	n.d.	20	1	1
Equivalent weight	1225	475	770	930	840	4,400
Hence uronic anhydride (%)	15	37	23	19	21	4
Acetyl (%)	2.5	0	0	0	0	0
Sugar composition after hydrolysis						
4-0-Methylglucuronic acid	2	2	0	1.5	0	0
Glucuronic acid	8	13	15	17.5	21	4
Galacturonic acid	5	22	8	0	0	0
Galactose	36	50	50	39	77	69
Arabinose	45	5	15	28	2	27
Rhamnose	4	8	12	14	0	0

* Data from Anderson and Stoddart (1966); Anderson et al (1967).

DISCUSSION

Combretum gums are always more viscous (Anderson et al, 1986) than gum arabic; this, and their acetyl content, are readily identifiable analytical characteristics. An additional simple differentiation can be obtained by placing powdered gum arabic and gum combretum, in separate dishes, inside a closed vessel containing some water to create a high humidity. The combretum gum will turn overnight into a sticky mass, showing its much more hygroscopic nature which is the cause of the "blocking" that occurs in storage or if gum combretum is used, for example, to make gummed paper for labels, envelopes, etc. Yet another unique analytical parameter is the presence of galacturonic acid in *Combretum nigricans* gum, which also has low nitrogen and rhamnose contents and a high arabinose content.

TABLE 2
Methyl Glycosides (%) in the methanolysates of *Combretum nigricans* gum and its degradation products.

Methyl Glycoside	Original Gum	Degraded Polysaccharides A	Degraded Polysaccharides I	
2,3,4-tri-O-Mē-L-Rh	2.6	0.2	0.8	end groups
2,3,5-tri-O-Mē-L-Ara	12.0	8.9	7.3	
2,3,4-tri-O-Mē-L-Ara	2.0	–	0.6	
2,3,4,6-Tetra-O-Mē-D-Gal	18.7	9.6	15.9	
3,4-Di-O-Mē-L-Rh	8.0	3.2	9.5	intra-chain
3,5-Di-O-Mē-L-Ara	2.5	0.9	–	
2,5-Di-O-Mē-L-Ara	2.1	0.7	0	
2,4-Di-O-Mē-L-Ara	17.9	1.7	6.6	
2,3,6-tri-O-Mē-D-Gal	8.2	8.8	17.6	
2,3,4-tri-O-Mē-D-Gal	2.9	23.6	5.9	
3-O-Mē-L-Rh	2.9	4.6	2.9	branch points
2,3-Di-O-Mē-D-Gal	9.4	16.6	16.1	
2,3,4-tri-O-Mē-D-GlucA	9.6	8.0	9.7	end groups
2,3,4-tri-O-Mē-D-GalA	2.0	3.5	2.7	
2,3-Di-O-Mē-D-GlucA	1.0	0.8	2.2	intra-chain
2,3-Di-O-Mē-D-GalA	2.3	8.7	2.2	

None of these superficial analytical differences explain, however, the great differences in functionality between gum arabic and *C. nigricans* gum. Evidence from the classical degradation methods used in structural studies of polysaccharides is necessary; Table 1 reveals the great differences between *C. nigricans* gum and gum arabic when they are degraded in two different ways.

The long-established structural features of gum arabic are that all of its rhamnose residues are chain-terminal and are joined to glucuronic acid and that these two sugars occupy peripheral sites in the globular, highly branched, gum macromolecules (Anderson et al, 1967). On acidic degradation, degraded gum arabic essentially contains only galactose and glucuronic acid; all rhamnose and almost all arabinose units are eliminated. On periodate oxidation, the arabinose and galactose units are largely resistant but all of the rhamnose and most of the glucuronic acid are eliminated.

Combretum nigricans gum behaves in a very different way. Both acidic and periodate degradations lead to the isolation of degraded

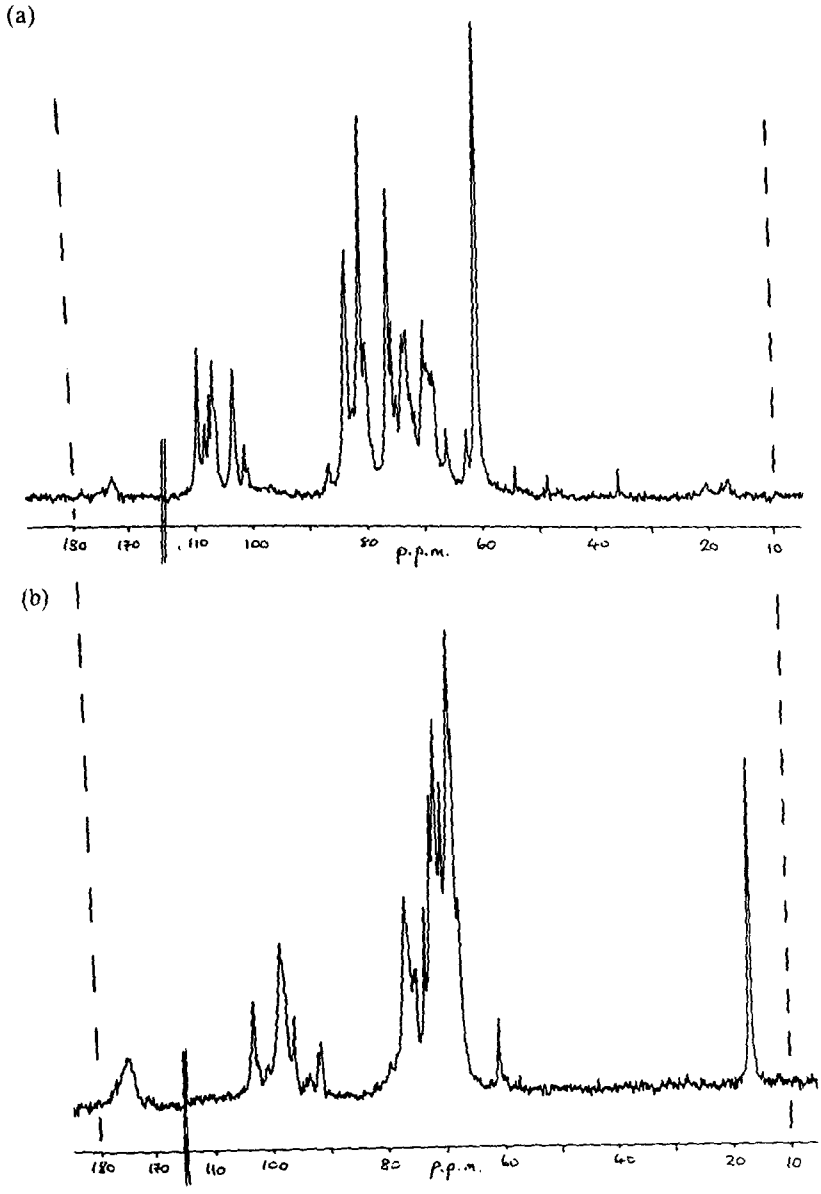

Figure 1. (a) ^{13}C-NMR spectrum for *C. nigricans* gum; (b) ^{13}C-NMR spectrum for its degraded polysaccharide A. Resonance for rhamnose at 16.5 ppm; resonance for uronic acid at 175 ppm.

polysaccharides, in 30–35% yield, in which there is depletion of arabinose (as in gum arabic) but in which there are considerably increased proportions of rhamnose, glucuronic acid and galacturonic acid. The major features of *C. nigricans* gum are, therefore: (a) most of its arabinose is peripheral, together with some galactose (b) significant proportions of its rhamnose and uronic acids are not readily degradable, i.e. they are not peripheral but are involved in the inner core of the branched molecular structure.

The extent of the enrichment of uronic acids (175 ppm) and particularly of rhamnose (16.5 ppm) in the acid-degraded product from *C. nigricans* gum is strikingly illustrated by the ^{13}C-N.M.R. spectra shown in Figure 1.

The structures of gum arabic and *C. nigricans* are therefore fundamentally different and this is reflected in their different functionality. Thus *C. nigricans* gum is not capable of being used as a replacement for gum arabic. These conclusions for *C. nigricans* gum are substantiated in the much more complex structural information resulting from classical methylation analysis (Table 2). This shows that small proportions of rhamnose and uronic acids are present as end groups, but that most of the rhamnose and uronic acids occupy intra-chain positions within the branched core of the complex gum molecules. Examination of the aldobiuronic acids isolated showed that at least some of the rhamnose and galacturonic acid are directly linked to each other.

The complex nature of the genus *Combretum* can now be illustrated by comparisons of these conclusions with those of previous studies on *C. leonense* gum (Aspinall and Bhavanandan, 1965) and *C. hartmannianum* gum (Anderson and Bell, 1976); *Combretum nigricans* has features in common with *C. leonense* but these species appear to differ from *C. hartmannianum*. Thus *C. nigricans* and *C. leonense* contain the same aldobiuronic acids and the majority of their uronic acids are intra-chain. But, *C. hartmannianum* has galacturonic acid linked to mannose, not to rhamnose, and all of its uronic acids and rhamnose are present as end-groups. All three gums have large proportions of peripheral arabinose; this is largely in the pyranose form in *C. leonense* and *C. hartmannianum*, but, in contrast, is largely in the furanose form in *C. nigricans* gum.

For years the properties of the Combretum gums, most commonly met in commerce as gum of West African origin from *C. nigricans*, have not been clearly understood and hence not marketed to best

advantage. Because of their fundamental structural differences, gum combretum and gum arabic have different functional characteristics. No Combretum gum is permitted in foodstuffs: the much tighter specification for gum arabic (FAO, 1990) means that parcels of gum offered for sale cannot be bought for food use, at top prices, if they are admixtures of gum arabic with *C. nigricans* gum. Toxicological evidence of safety for a plant gum takes at least five years to establish at a cost of at least a million US dollars. Even if this level of cash flow were to be available, it appears to be highly unlikely that *C. nigricans* gum would be toxicologically acceptable because of its tannin content, and the presence of unacceptably high levels of other impurities, colouring pigments, and bark extractives which are potent pharmacologically (Petitt et al, 1988; Singh and Petitt, 1989). Large amounts of *C. nigricans* gum are, nevertheless, potentially available and marketing strategies could be devised to optimise commerical benefits, in recognition of the fact that the gum's unique structure leads to some technological properties which allow it to outperform gum arabic in some applications.

For many years, *C. nigricans* has commanded the lowest demand and hence price of all of the exudate gums. Greater attention to locating natural production areas (as around Sokoto) for top quality Combretum gum of the palest possible colour, complete avoidance of dark red or fire-blackened gum, and marketing strategies aimed at keeping *C. nigricans* gum as a separate, well-defined commodity available in regular supply and constant quality, avoiding at all costs its use to adulterate gum arabic, would lead to greater demand and generation of a larger cash flow. The major understanding must, however, be the great susceptibility of *C. nigricans* gum to moisture, and hence the need for greater marketing consideration to be given to its storage and transport, especially during the rainy season, and to the possibility of packing it in plastic bags rather than old jute sacks.

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RÉSUMÉ

Combretum nigricans est la source principale de la "gomme combretum" commerciale dont l'on trouve beaucoup sur les marchés de l'Afrique occidentale. Quoique les autorités ne la permettent pas dans les comestibles, l'on s'en sert pour frélater la gomme soudanaise (*Acadia senegal*), avec des résultats malheureuses puisque cela réduit nettement les propriétés souhaitables de cette dernière. La gomme combretum elle-même a une mauvais réputation d'autocollage, formant ainsi la gomme "bloquée", quoiqu'elle ait certaines applications commerciales si elle est conservée et employée convenablement. La spectrométrie de résonance magnétique ^{13}C nucléaire et des procédures de dégradation ont montré la différence essentielle de structure chimique dont il est question: les éléments constitutifs de rhamnose et d'acide uronique, qui occupent des positions structurales périphériques terminant la chaîne, dans la gomme soudanaise, se trouvent dans des positions internes dans la gomme combretum.

RESUMEN

El árbol *Combretum nigricans* es la fuente más importante de la "goma combretum" comercial que se halla disponsible abundantemente en los mercados del Africa Occidental. Aunque las autoridades reguladoras no la permiten en los productos alimenticios, se ha usado como ingrediente adulterante de la goma arábiga (*Acacia senegal*) con resultados perjudiciales porque las propiedades deseables de ésta última se reducen notablemente con ella. La goma combretum misma tiene una desafortunada reputación por ser autoadherente y formar goma "bloqueada", aunque es deseable para algunas aplicaciones comerciales, si se almacena y usa apropiademente. La espectrometria de resonancia magnética nuclear del ^{13}C y los procedimientos degradantes han demostrado la diferencia esencial de sus estructural quimicas: la diferencia esencial de sus estructuras quimicas: la rhamnosa constituyente y las unidades de ácido urónico, que ocupan posiciones estructurales periféricas en los terminales en cadena de la goma arábiga, en la goma combretum están en posiciones situadas en el interior.

FOOD ADDITIVES AND CONTAMINANTS, 1991, VOL. 8, NO. 4, 405–421

Gum arabic (*Acacia senegal*): unambiguous identification by ^{13}C-NMR spectroscopy as an adjunct to the Revised JECFA Specification, and the application of ^{13}C-NMR spectra for regulatory/legislative purposes

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The JECFA Specification for gum arabic was revised in 1990 to reflect more closely the specification of the Test Article used in evaluations that led to its classification 'ADI not specified' in 1982/83. Some producers and traders have objected to the Revised Specification; in contrast, consumer-protection groups consider that it remains too lax to provide the degree of safety assurance expected. This paper presents analytical data that confirm the mean values previously established for nitrogen and the specific rotation of bulk commercial gum arabic from *Acacia senegal*. The data also establish that natural gum arabic imported into the USA and Europe in 1989/91 met the Revised Specification, but that a disturbingly high proportion of spray-dried, processed gums sold as 'gum arabic' did not. NMR spectroscopy has (a) indicated that some such samples are based on non-permitted gums and (b) confirmed that the 1983 Test Article represents not only typical 1990/91 shipments but also a wide range of reference gum arabic samples from other reputable sources. Details of a representative ^{13}C-NMR spectrum, derived by averaging the relative intensities for the characteristic resonances of 35 gum arabic samples, are given for future regulatory/legislative purposes. Some limitations of the Revised Specification and its susceptibility to commercial exploitation are discussed.

Keywords: Gum arabic, revised specification, *Acacia senegal*, NMR spectra, gum talha, gum combretum.

Introduction

Following requests (WHO 1974), toxicological evidence of safety for gum arabic (*Acacia senegal*) was provided from several sources. The studies involved, which have been reviewed (Anderson 1986, Anderson and Eastwood 1989), led to the classification 'ADI not specified' by JECFA (FAO 1982). Details of the Test Article used, e.g. in a dietary study in Man (Ross *et al.* 1983), allergenicity tests (Strobel *et al.* 1982), animal-based studies (Anderson *et al.* 1982) and in studies of tissues by transmission electron microscopy (Anderson *et al.* 1986), were published (Anderson *et al.* 1983), together with details of how the Test Article had been selected with great care by sponsors in order to ensure that it could be accepted as a representative sample of bulk commercial gum arabic of 'fair average quality'. The Test Article complied in every respect with all of the regulatory specifications for identity and purity in existence at the time of its adoption in 1980. Subsequently, a revision by JECFA (FAO 1986) omitted the test requiring a negative specific rotation. This, it is now known, led quickly to complaints, from large users of gum arabic, that gums other than that defined were being supplied (WHO 1990a,b), resulting in unsatisfactory products.

0265-203X/91 $3.00

Table 1. Analytical data for natural gum arabic samples provided by importers in 1990/91.

Sample No.	Sample sent by	Date received	% H_2O	% Ash	% N	Specific rotation[a] (degrees)	Revised JECFA Spec. (1990)	Confirmation from NMR spectrum
N1	American importer A	Dec. 1990	13·2	3·8	0·34	−29	Conforms	Good *Acacia senegal*
N2	American importer A	Dec. 1990	13·9	4·0	0·36	−31	Conforms	Good *Acacia senegal*
N3	Italian importer B	Dec. 1990	14·4	3·3	0·31	−30	Conforms	Good *Acacia senegal*
N4	British importer C	Dec. 1990	14·5	3·6	0·37	−31	Conforms	Good *Acacia senegal*
N5	British importer D	Dec. 1990	12·2	3·5	0·35	−33	Conforms	Good *Acacia senegal*
N6	British importer E	Dec. 1990	14·9	2·9	0·38	−33	Conforms	Good *Acacia senegal*
N7	German importer F	Jan. 1991	14·4	4·0	0·28	−34	Conforms	Good *Acacia senegal*
N8	American importer G	Jan. 1991	15·0	3·2	0·29	−26	Conforms	Good *Acacia senegal*
N9	American importer H	Jan. 1991	13·9	3·9	0·33	−32	Conforms	Good *Acacia senegal*
N10	British importer K	Feb. 1991	13·3	3·7	0·34	−28	Conforms	Good *Acacia senegal*
N11	Italian importer L	Feb. 1991	14·8	3·4	0·30	−29	Conforms	Good *Acacia senegal*
Mean values			14·0	3·6	0·33	−30·5		

[a] Dry-weight basis, as specified (FAO 1990).

All samples conformed to the Revised (1990) JECFA Specification in respect of solubility (complete in cold water); acid-insoluble ash (> 0·5%) and matter (> 1%); starch/dextrin (absent); tannin (absent); arsenic (> 3 ppm), lead (> 10 ppm), heavy metals (> 40 ppm).

As a result, the evidence of safety for gum arabic was reviewed by JECFA in 1989; this led to the publication of three reports in 1990.

(a) The first (WHO 1990a) evaluated the additional toxicological evidence submitted after the previous evaluation in 1982, viz. reports sponsored by the International Natural Gums Association for Research Ltd (INGAR), the UK Ministry of Agriculture, Fisheries and Food, and the American FDA. The specification for the Test Article (Anderson *et al.* 1983) was referred to in some detail and gum arabic was defined as 'the dried gummy exudate from *Acacia senegal* trees'.

(b) The second (WHO 1990b) stated that JECFA's attention had been 'drawn to the fact that products were being sold as gum arabic that were derived from species other than *Acacia senegal* and closely related species hitherto recognized as the source species of gum arabic'. The existing specifications (FAO 1986)

Table 2. Analytical data for processed gum arabic samples received 1989–90.

Sample No.	Country of origin of User	Country of origin of Supplier	% H_2O	% Ash	% N	Specific rotation[a] (degrees)	Revised JECFA Spec. (1990)	Confirmation from NMR spectrum
P1	USA	USA	6·5	3·2	0·31	−31	Conforms	Good *Acacia senegal*
P2	USA	France	9·6	3·4	0·36	−28	Conforms	Good *Acacia senegal*
P3	USA	Germany	11·2	3·4	0·35	−30	Conforms	Good *Acacia senegal*
P4	UK	UK	7·1	3·0	0·30	−26	Conforms	Good *Acacia senegal*
P5	France	Not disclosed	7·7	3·4	0·37	−32	Conforms	Good *Acacia senegal*
P6	France	Not disclosed	8·0	3·4	0·36	−34	Conforms	Good *Acacia senegal*
P7	France	Not disclosed	8·1	3·2	0·35	−30	Conforms	Good *Acacia senegal*
P8	France	Not disclosed	7·8	3·0	0·34	−34	Conforms	Good *Acacia senegal*
P9	France	Not disclosed	8·2	3·5	0·36	−29	Conforms	Good *Acacia senegal*
P10	France	Not disclosed	7·8	3·0	0·37	−32	Conforms	Good *Acacia senegal*
P11	France	Not disclosed	8·3	2·9	0·32	−32	Conforms	Good *Acacia senegal*
P12	France	Not disclosed	8·0	3·5	0·28	−34	Conforms	Good *Acacia senegal*
Mean values			8·2	3·2	0·34	−31		

[a] Dry-weight basis, as specified (FAO 1990).

All samples conformed to the Revised (1990) JECFA Specification in respect of solubility; acid-insoluble ash (> 0·5%) and matter (> 1%); starch/dextrin (absent); tannin (absent); arsenic (> 3 ppm), lead (> 10 ppm), and heavy metals (> 40 ppm).

were therefore revised 'to reflect more closely the gums that had been toxicologically evaluated'.

(c) The third (FAO 1990) gives the Revised Specification, which differs in only the following three respects from that superseded:
  (i) Gum arabic is now defined as originating from '*Acacia senegal* (L.) Willd. or closely related species'—the difference involving simply the insertion of the word 'closely'.
  (ii) A permitted range (−26 to −34 degrees, calculated on a dry-weight basis) is now quoted for the specific rotation.
  (iii) A permitted range for the Kjeldahl nitrogen content (0·27 to 0·39%) is now specified (this range would be less ambiguous if a dry-weight basis were to be specified, as for the specific rotation).

The Revised Specification is easily met by the 1982 Test Article and by 35 Sudanese and Nigerian gum arabic samples collected between 1904 and 1989

Table 3. Analytical data for some processed 'gum arabic' samples not in compliance with Specifications, 1989/90.

Sample No.	Country of origin of Complaint	Country of origin of Supplier	% H_2O	% Ash	% N	Specific rotation[a] (degrees)	Tannins	Revised JECFA Spec. (1990)	Confirmation of origin by NMR spectrum
F1	USA	USA	8·4	3·3	0·15	+22	−ve	Fail	Mixture (approx· 50/50) of gums talha and arabic
F2	USA	Germany	9·1	3·5	0·18	−15	−ve	Fail	Not gum arabic—'white Mali gum' (talha/combretum mixture)
F3	USA	France	8·1	2·1	0·21	−34	+ve	Fail	Not gum arabic—gum combretum
F4	Belgium	France	10·8	2·3	0·22	−53	+ve	Fail	Not gum arabic—gum combretum
F5	Belgium	Germany	8·0	2·1	0·40	−17	+ve	Fail	Not gum arabic—gum combretum/talha
F6	Belgium	Germany	9·0	3·4	0·40	−11	+ve	Fail	Not gum arabic—gum combretum/talha
F7	France	Not disclosed	8·7	3·6	0·34	−6	−ve	Fail	Not gum arabic—West African combretum + *Acacia polyacantha* mixture
F8	France	Not disclosed	8·4	3·7	0·38	−10	−ve	Fail	Not gum arabic—West African combretum + *Acacia polyacantha* mixture
F9	Holland	Not disclosed	7·5	3·5	0·18	+12	+ve	Fail	Not gum arabic—West African talha/combretum mixture

[a] Dry-weight basis, as specified (FAO 1990).

(Anderson *et al.* 1990). Nevertheless, adverse comments were submitted by some gum producers and traders for consideration at the meeting of the Codex Committee on Food Additives and Contaminants held in March 1991, requesting either reversion to the unsatisfactory specification of 1986, or an extension of time—seven years after the JECFA classification 'ADI not specified' and publication of the Test Article's specification—'to permit measures to be taken to ensure compliance'. In contrast, consumer interests expressed disappointment that the Revised Specification failed to ensure safety for consumers because its few identification and purity tests can be met by non-approved natural gums from botanical sources other than that now defined for gum arabic.

Table 4. Analytical data (Revised Specification) for reference gum arabic samples, 1904–91.

Sample No.	Sample provided by	Country and season of origin	% H_2O	% Ash	% N	Specific rotation[a] (degrees)	Revised JECFA Spec. (1990)
R1	Tropical Products Institute, London	Sudan 1904	14	3·8	0·38	−32	Conforms
R2	TPI London	Sudan 1905	14	3·9	0·34	−31	Conforms
R3	Rowntree, UK	Sudan 1960	13	3·7	0·32	−32	Conforms
R4	TPI London	Nigeria 1967	12	3·9	0·29	−29	Conforms
R5	Gum research officer	Sudan 1970	13	3·4	0·31	−31	Conforms
R6	French importer	Sudan 1971	13	3·4	0·38	−31	Conforms
R7	Sonimex, Nouakchott	Mauritania 1988	14·8	4·0	0·30	−26	Conforms
R8	Mr I. Holmes	Kenya 1989	15·0	2·8	0·35	−33	Conforms
R9	Mr V. Minerate	Chad 1990	15·0	2·9	0·35	−33	Conforms
R10	District Officer, Karamoja	Uganda 1990	14·2	3·2	0·27	−32	Conforms
R11	Principal agroforester	Niger 1990	12·4	2·6	0·30	−34	Conforms
R12	INGAR Test Article	General 1982	(6·0)[b]	3·0	0·31	−30	Conforms
Mean values			14	3·4	0·33	−31	

[a] Dry-weight basis, as specified (FAO 1990).
[b] Spray-dried sample.

Because of the consequent immediate regulatory interest in the extent of the justification for some trade claims that 1990/91 gum season shipments may not be in compliance with the Revised Specification, this study was initiated in December 1990 in response to a request for a further contribution to the International Programme on Chemical Safety.

Experimental

Origins of gum samples

Eleven samples (N1–N11, table 1) were supplied by prominent gum importers in Germany, Italy, the UK and the USA between December 1990 and January 1991,

Table 5. Resonance (ppm) and relative intensity data for ^{13}C Fourier-transform NMR spectra of natural gum arabic samples, 1990/91.

Resonance (ppm) (± 0·2)	Relative intensities Samples[a]											Mean ($n = 11$)	sd
	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11		
175·0	0·7	1·58	2·16	1·90	1·04	0·7	1·56	1·43	1·40	2·21	1·42	1·45	0·51
109·5	5·79	4·38	4·65	5·50	4·78	4·89	5·33	5·30	4·31	5·42	4·98	5·03	0·48
108·1	3·25	3·16	3·08	3·77	3·35	3·11	3·37	3·28	2·62	4·00	3·25	3·29	0·36
102·9	5·94	7·43	6·58	7·46	7·07	7·57	7·10	6·57	6·00	6·39	5·94	6·73	0·63
100·6	9·47	10·27	9·78	10·36	9·72	10·76	8·52	9·75	8·64	8·27	9·48	9·55	0·79
100·0	5·51	6·07	5·99	6·72	6·23	5·06	5·02	5·60	5·61	6·55	6·01	5·85	0·55
99·1	2·11	2·40	2·21	1·91	1·70	1·41	1·17	1·22	1·80	1·85	2·32	1·83	0·42
84·8	3·19	3·00	3·88	3·60	3·65	3·05	3·17	3·46	3·56	3·56	3·36	3·41	0·28
83·9	4·83	4·50	5·41	5·06	4·90	4·44	4·46	5·07	4·68	5·02	4·66	4·82	0·31
82·8	5·67	5·53	5·63	5·58	5·57	4·16	5·25	5·41	5·57	5·70	5·05	5·37	0·45
81·3	7·66	7·11	6·92	7·72	7·46	7·77	7·40	7·70	6·20	7·75	6·41	7·28	0·56
80·1	5·92	6·30	6·10	5·79	6·29	4·90	5·71	5·97	5·88	5·50	5·09	5·77	0·45
79·0	7·77	9·66	9·00	9·02	9·25	9·36	8·28	8·83	8·57	7·31	8·01	8·64	0·73
76·7	5·51	5·00	5·53	5·64	5·00	5·10	4·87	5·89	5·18	5·34	4·75	5·25	0·35
76·0	6·90	10·99	9·75	8·36	9·08	9·68	8·77	8·76	8·42	4·17	7·09	8·36	1·81
74·1	10·86	11·83	10·95	11·11	11·05	11·84	10·15	11·68	10·64	9·23	9·93	11·00	0·82
73·2	12·92	13·77	12·54	13·47	12·67	14·06	12·14	13·53	12·13	10·95	12·26	12·77	0·91
71·9	10·56	13·90	12·53	11·22	11·25	11·75	10·36	11·64	12·26	9·51	10·35	11·39	1·22
71·3	8·49	11·09	9·98	9·13	10·06	7·85	8·53	8·63	10·14	9·30	8·65	9·26	0·96
70·2	14·67	17·80	17·49	15·02	17·00	15·67	14·95	15·62	n.r.	14·56	13·34	15·61	1·42
69·9	16·96	20·30	18·73	17·51	18·38	18·73	16·80	17·82	17·05	15·08	16·04	17·58	1·44
69·1	18·11	21·45	18·83	17·65	19·43	16·10	17·26	17·93	19·22	18·43	17·90	18·39	1·37
68·9	15·77	21·40	17·70	17·46	19·10	16·35	16·71	17·23	18·39	16·17	15·24	17·41	1·74
68·2	9·95	11·20	10·67	10·03	11·00	8·52	10·17	9·90	10·83	10·92	9·36	10·23	0·80
63·2	2·00	1·90	2·49	2·12	1·95	1·34	1·65	2·10	1·70	2·07	2·00	1·94	0·30
61·2	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	
16·5	4·90	8·04	9·42	8·71	6·35	5·93	8·13	9·94	9·91	5·35	6·83	7·59	1·82
Factor	×0·96	×0·96	×1·13	×0·89	×0·96	×0·86	×1·0	×0·96	×0·97	×1·0	×0·96		

[a] Samples numbered as in table 1.
n.r. = not resolved.

Table 7. Resonance (ppm) and relative intensity data for ^{13}C Fourier-transform NMR spectra of reference gum arabic samples.

Resonance (ppm) (± 0·2)	Relative intensities Reference samples[a] R1 1904	R2 1905	R3 1960	R4 1967	R5 1970	R6 1971	R7 1988	R8 1989	R9 1990	R10 1990	R11 1990	Test Article R12 1982	Tables 5,6,7 Mean (n = 12)	sd	Grand mean (n = 35)	sd
175·0	1·80	2·16	1·53	3·59	1·62	0·95	1·69	2·79	1·02	2·42	1·75	1·79	1·93	0·74	1·84	0·72
109·5	5·06	5·78	4·17	4·92	4·70	6·09	4·41	5·78	5·29	5·03	6·11	5·10	5·20	0·63	5·20	0·53
108·1	3·55	3·99	2·76	3·97	3·21	4·28	3·10	3·77	3·05	3·30	3·72	3·25	3·50	0·46	3·41	0·40
102·9	7·35	7·69	6·75	7·77	6·20	6·27	7·25	6·53	8·23	6·90	7·11	6·40	7·05	0·65	6·90	0·59
100·6	9·69	10·07	9·33	10·33	7·53	7·12	10·15	11·18	12·30	9·04	10·10	9·13	9·66	1·42	9·70	1·02
100·0	5·92	6·59	5·41	6·72	4·79	5·06	5·50	6·65	5·05	6·07	5·99	5·94	5·81	0·65	5·95	0·62
99·1	1·80	2·00	2·00	1·92	1·72	1·96	1·50	1·48	1·75	1·70	2·28	2·27	1·87	0·26	1·90	0·38
84·8	4·20	4·84	2·75	3·84	3·26	3·47	3·56	4·02	3·32	3·35	3·33	3·42	3·61	0·54	3·48	0·39
83·9	5·84	5·98	3·79	5·28	5·13	7·82	5·90	5·45	5·19	5·20	5·77	4·97	5·52	0·93	5·06	0·60
82·8	6·39	6·75	4·89	6·97	5·43	5·61	6·26	5·79	4·76	5·27	5·30	5·63	5·75	0·70	5·61	0·52
81·3	7·99	8·20	7·23	7·60	7·46	11·23	8·16	9·59	8·26	7·22	8·32	7·13	8·20	1·17	7·80	0·78
80·1	7·00	7·22	4·86	7·20	5·52	6·63	6·82	5·60	4·98	5·51	5·74	5·51	6·05	0·87	5·93	0·60
79·0	9·43	9·76	8·66	10·13	7·65	7·44	10·06	9·07	10·76	9·02	8·61	8·82	9·12	0·98	8·74	0·76
76·7	6·90	7·00	5·21	6·24	5·42	8·34	7·23	6·16	5·24	4·82	6·36	5·00	6·16	1·07	5·61	0·66
76·0	10·46	11·08	8·50	11·03	8·48	7·35	10·62	10·81	9·30	8·00	8·46	8·19	9·36	1·36	8·83	1·34
74·1	12·07	12·29	10·72	12·74	9·55	9·63	12·43	11·41	13·62	10·03	11·19	10·35	11·33	1·31	11·07	0·86
73·2	14·00	14·02	13·93	14·39	11·27	12·25	13·96	13·22	15·76	12·88	13·62	13·51	13·54	1·11	13·23	0·91
71·9	13·05	13·35	11·10	15·12	10·38	9·48	13·96	13·94	13·45	10·47	11·14	10·80	12·18	1·81	11·66	1·24
71·3	10·93	11·63	8·55	9·60	9·09	8·06	11·72	10·22	8·15	8·63	8·45	9·13	9·51	1·31	9·37	1·07
70·2	19·58	19·99	15·00	20·28	15·42	13·93	19·84	16·56	17·56	15·15	14·83	14·83	16·91	2·40	16·07	1·73
69·9	21·60	22·11	18·34	n.r.	16·37	14·34	n.r.	19·33	21·44	17·73	17·09	17·39	18·57	2·53	17·76	1·92
69·1	20·60	20·11	19·04	23·09	18·59	16·47	20·87	19·55	16·63	17·48	17·19	18·74	19·03	1·96	18·71	1·60
68·9	13·20	13·00	17·98	15·17	17·11	14·96	18·88	17·50	18·43	18·25	16·60	17·52	16·55	2·00	17·15	1·67
68·2	n.r.	n.r.	10·97	n.r.	10·00	10·16	10·60	11·98	9·04	11·53	10·51	11·18	10·66	0·88	10·52	0·94
63·2	2·30	3·55	2·00	n.r.	1·28	2·30	2·27	1·48	1·66	2·77	1·72	2·14	2·13	0·61	2·09	0·48
61·2	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11	25·11		25·11	
16·5	8·77	9·71	10·69	11·16	7·47	7·84	11·00	10·30	6·90	5·47	10·38	9·76	9·12	1·82	7·84	1·77
Factor	×1·0	×1·0	×1·0	×0·96	×1·0	×0·94	×1·0	×1·0	×1·55	×1·0	×0·86	×0·96				

[a] Origin of samples as in table 4.
n.r. = not resolved.

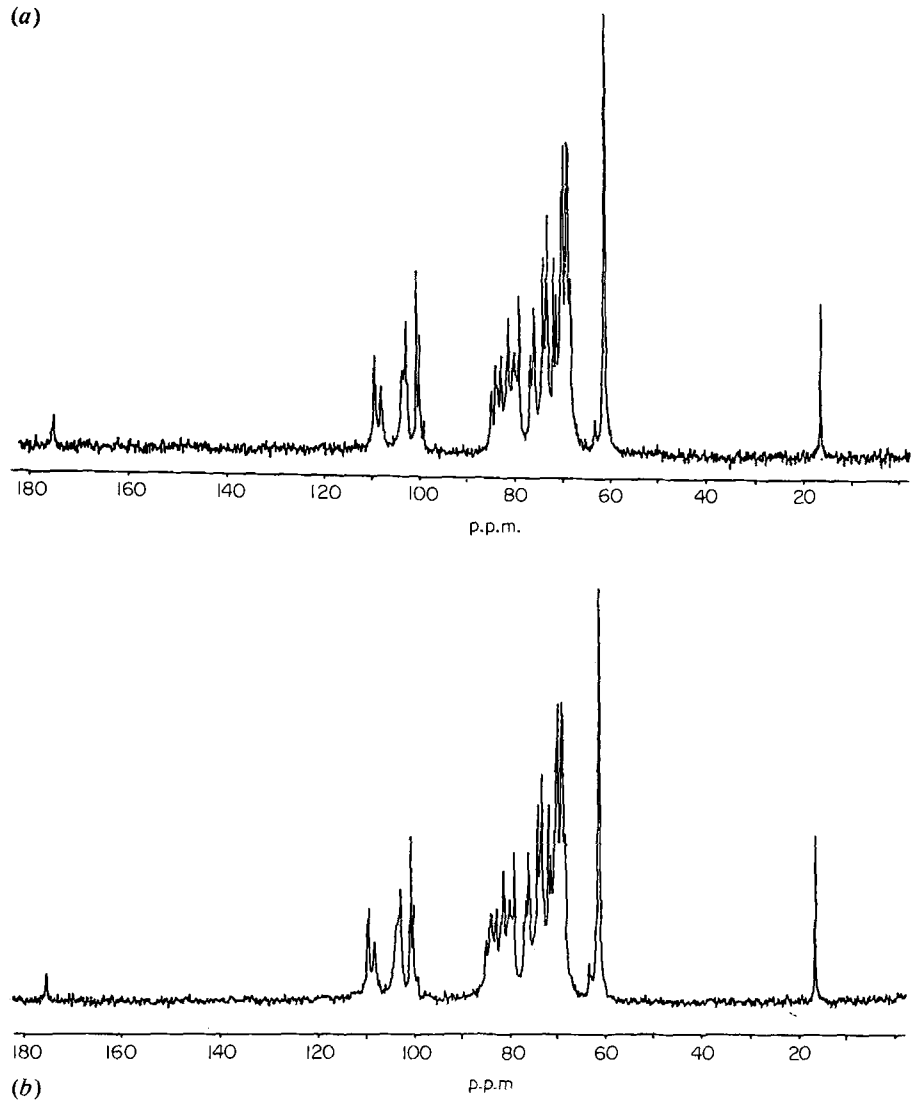

Figure 1. (a) ^{13}C-NMR spectrum for sample N5, UK importer. (b) ^{13}C-NMR spectrum for sample N8, USA importer.

representing the latest shipments of natural gum arabic received. Twenty-one samples of processed 'gum arabic' had been received in 1990, for purposes of referee confirmation of identity, from gum users in Belgium, France, Holland, UK and USA; information concerning the identity of the gum supplier was given in some cases but was not requested. Of these, twelve samples (P1–P12, table 2) conformed to the Revised Specification; nine samples (F1–F9, table 3) did not. Samples R1–R11 (table 4) are reference samples of gum arabic; some (R1–R6) from much earlier gum seasons for which full analytical data have been published (Anderson *et al.* 1990), and some (R7–R11) from current and immediate past seasons to give, in addition, a wide representative range of producing countries.

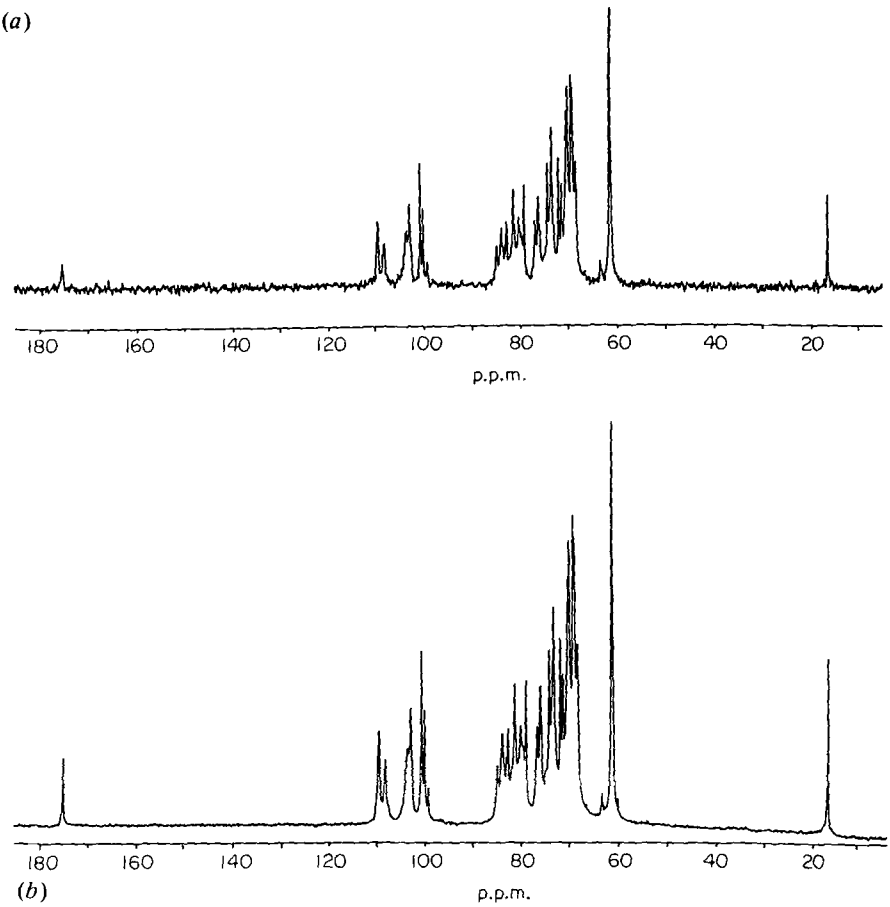

Figure 2. (a) ^{13}C-NMR spectrum for sample P3, German supplier. (b) ^{13}C-NMR spectrum for sample P8, French user.

Thus samples R1–R6 correspond to samples S1 (1904), S2 (1905), S5 (1960), N9 (1967), S8 (1970) and S9 (1971), the origins of which have been given (Anderson *et al.* 1990). Sample R7 was supplied by Messrs Sonimex, Mauritania, in 1988; Sample R8 was collected in Kenya by Mr I. Holmes in 1989; Sample R9 was collected in Chad by Mr V. Minerate in 1990; Sample R10 was collected in Karamoja Region, Uganda, by the District Forest Officer in 1990; Sample R11 was collected at Sadoré, Niger, by Dr R. J. Van Den Beldt, Principal Agroforester, ICRISAT, Niamey, in December 1990. Sample R12 is the 1982 Test Article (Anderson *et al.* 1983).

Analytical methods

Conformity with the Revised Specification (FAO 1990) requires testing for solubility, acidity, precipitation by alcohol, absence of starch/dextrin and tannin; and determinations of loss on drying, total ash, acid-insoluble ash, acid-insoluble matter, arsenic, lead, heavy metals, nitrogen and specific rotation. These Identification and Purity Tests were carried out as stated in the Revised Specification or in the Guide to JECFA Specifications (FAO 1983), except for the

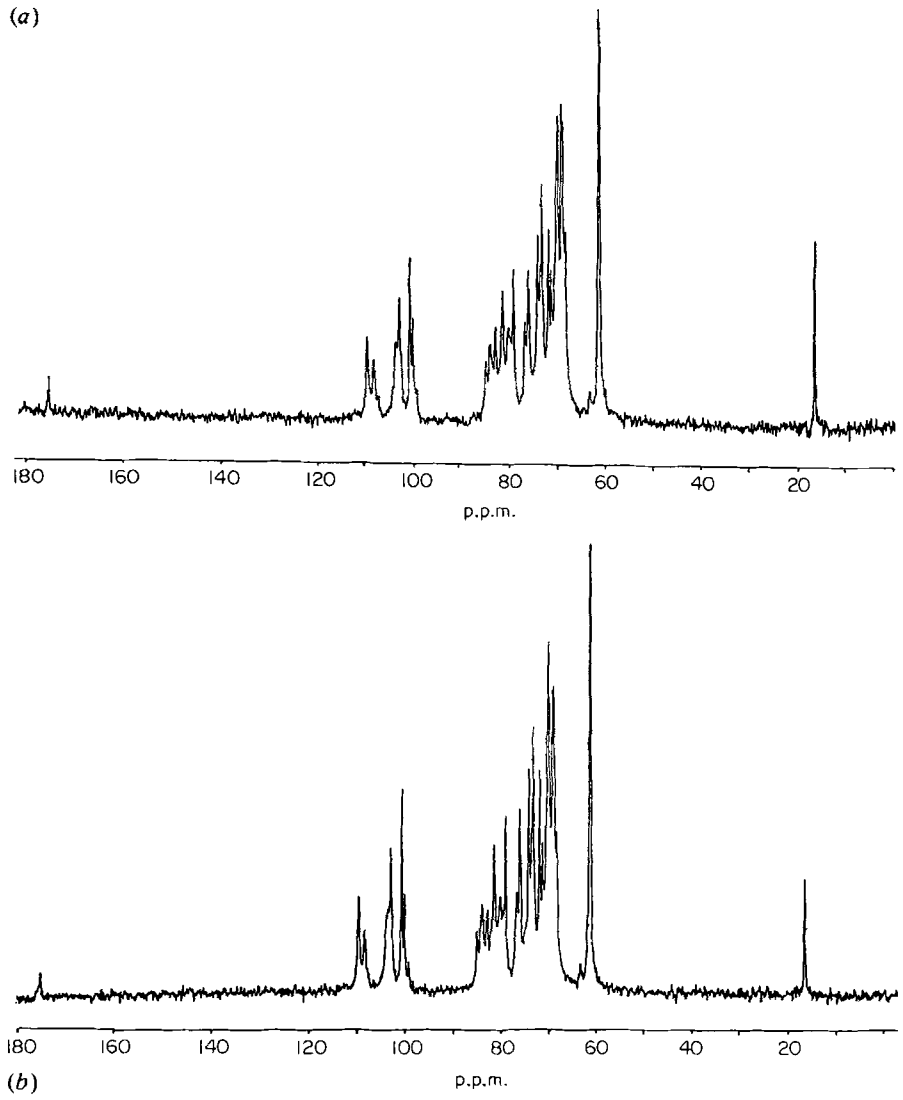

Figure 3. (a) ^{13}C-NMR spectrum for sample R3, 1960. (b) ^{13}C-NMR spectrum for sample R9, Chad 1990.

determination of specific rotation, which was made on 1% (w/w) aqueous solutions, not 10% as described (FAO 1990). However all specific rotation values in tables 1–4 are quoted on a dry-weight basis, as stated in the Revised Specification (FAO 1990).

^{13}C-Nuclear magnetic resonance spectrometry

^{13}C Fourier-transform NMR spectra for 10% gum solutions in D_2O at room temperature were recorded overnight at 50·320 MHz with a Brüker WP200 SY spectrometer. All spectra were recorded under identical instrumental operating conditions. To facilitate their direct comparison, the relative intensities recorded by the spectrometer for the sample solution, which differed slightly in some cases in

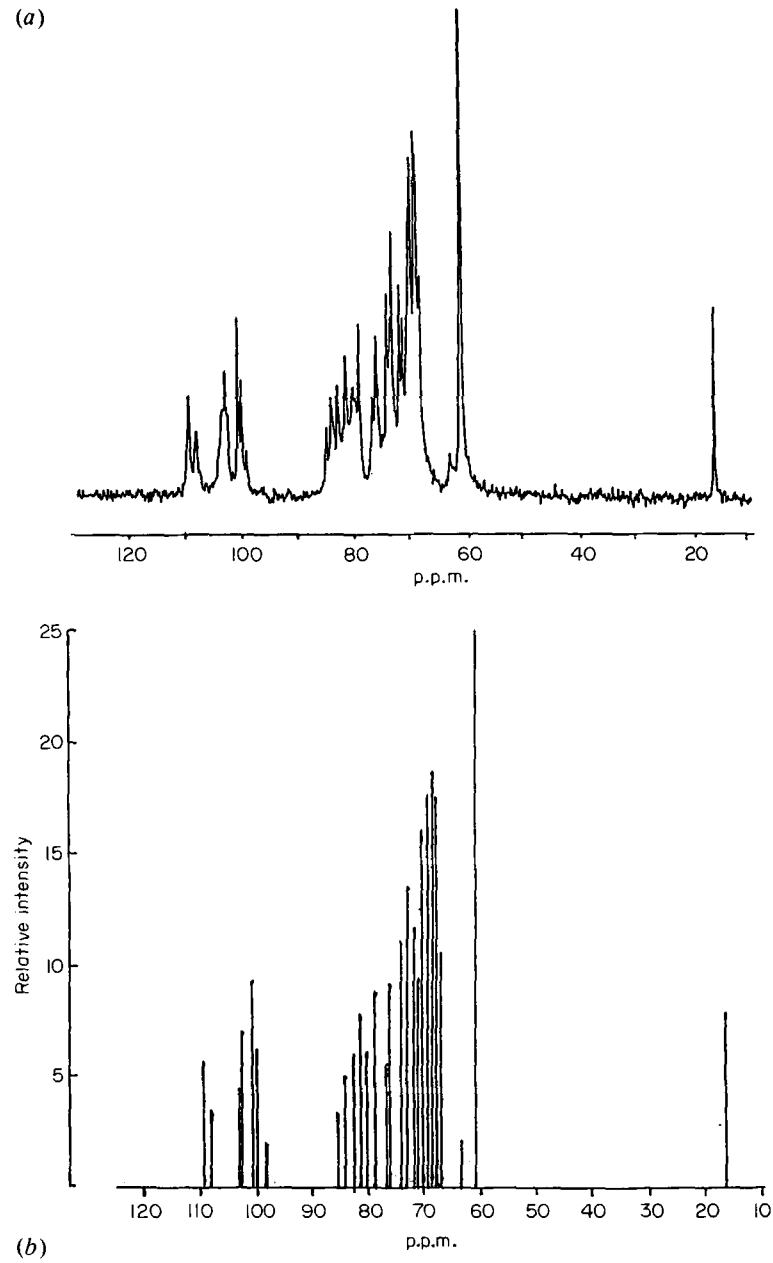

Figure 4. (a) ^{13}C-NMR spectrum for sample R12 (Test Article). (b) Representative ^{13}C-NMR spectrum for gum arabic (relative intensities are the means of those recorded for 35 samples (Tables 5, 6, 7)).

terms of their final concentrations (through filtration losses or presence of some insoluble gel-type material, etc.) were multiplied by the conversion factors shown in tables 5–7 to make the intensity of the principal resonance (61·2 ppm) equal for all samples.

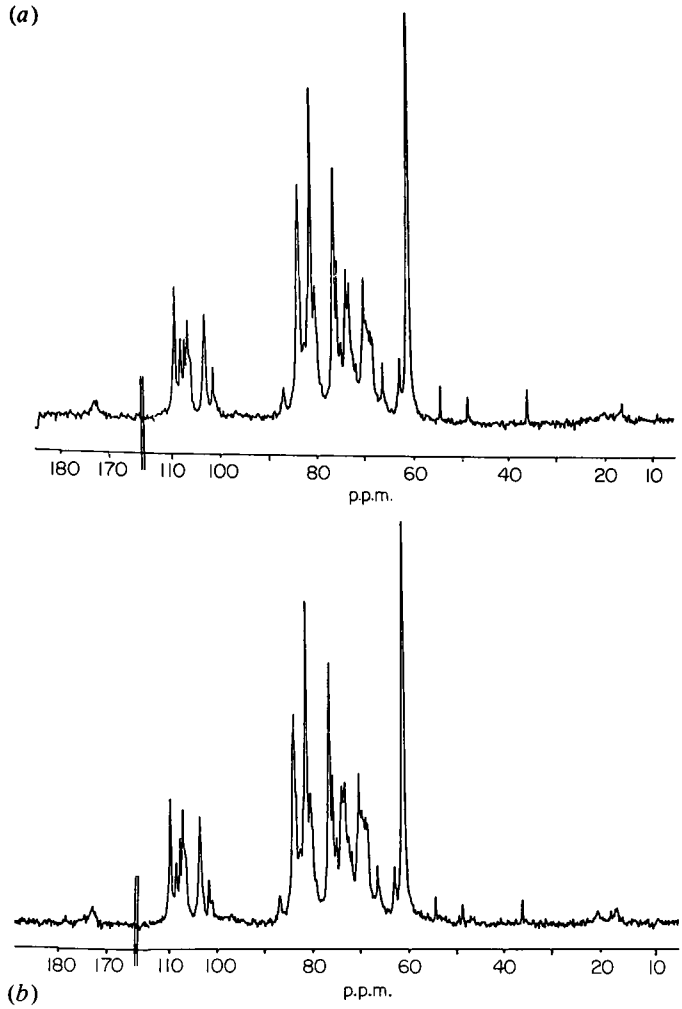

Figure 5. (a) Representative ^{13}C-NMR spectrum for *Combretum nigricans* gum. (b) ^{13}C-NMR spectrum for sample F3 (F4 spectrum virtually identical).

Results

Table 1 shows the data for natural gum arabic samples N1–N11 provided by international importers in 1990/91. Tables 2 and 3 show the data for the processed gum arabic samples received for evaluation from gum users in gum season 1989/90 and 1990/91. Samples P1–P12 (table 2) conform to the Revised Specification; samples F1–F9 (table 3) do not. Table 4 shows the data for the 1982 Test Article (sample R12) and other reference samples (R1–R11) selected from the large number now available for the period 1904–1990. Tables 5, 6 and 7 record the corresponding ^{13}C-NMR spectroscopy data for samples N1–N11, P1–P12 and R1–R12. To assist visual comparisons of the extent of the small fine-structural variations involved, representative spectra are shown in figure 1 (samples N5 and N8), figure 2 (samples P3 and P8), figure 3 (samples R3 and R9) and figure 4 (sample R12, the Test Article), and a representative ^{13}C-NMR spectrum for gum arabic from *Acacia*

senegal derived by averaging the intensities of the resonances for samples N1–N11, P1–P12 and R1–R12. Figure 5 shows that the spectrum for sample F3 (the spectrum for F4 is virtually identical) corresponds with that representative of *Combretum nigricans* gum; samples F3 and F4 were therefore falsely described as 'gum arabic' by their vendors.

Discussion

The mean values for the nitrogen content and specific rotation of each of the sub-sets of samples in tables 1, 2 and 4 agree excellently with the values (0·34% and −30 degrees respectively) established previously (Anderson *et al.* 1990) for $n = 35$.

The data in table 1 show that each of the random selection of American and European gum importers requested in the period November 1990–January 1991 to supply a sample of gum arabic, representative of their latest delivery from Africa, provided gum (samples N1–N11) that complied in all aspects with the Revised Specification. There is no reason to suspect that other merchants could not have done likewise had they been approached. It is, however, an unfortunate weakness of the Revised Specification that it does not serve to define gum arabic unambiguously and to the exclusion of other water-soluble exudates. As has been pointed out (Food Commission 1991) the Revised Specification could be met by untested gums, or blends of untested gums, from a widely available variety of alternative tropical trees. For this reason, samples N1–N11 were also examined by ^{13}C-NMR spectroscopy (table 5 and figure 1), which confirmed that all of these natural gum samples were gum arabic originating from *Acacia senegal*.

As all gum importers and experienced trade buyers of gum arabic know, sound judgement can usually be exercised in assessments of the identity/quality of natural gum arabic; the colour, odour, size and surface markings on the gum nodules are all strongly characteristic. The reliability of such visual assessments is greatly decreased, however, if the gum has been kibbled (mechanically broken into small fragments of a particular mesh size), powdered, or processed into a spray-dried, roller-dried, or freeze-dried version. For this reason some trade buyers insist upon inspecting the crude, natural gum before it is kibbled, powdered, or spray-dried to their order. This is reflected in the results obtained for the 21 samples of processed gum arabic that had been submitted voluntarily by trade users in several countries for independent assessment in gum seasons 1989/90 and 1990/91.

Twelve of these samples (P1–P12) complied with the Revised Specification (table 2) and gave ^{13}C-NMR spectra characteristic of good quality gum arabic (table 6 and figure 2). But nine samples (F1–F9) did not comply with the Revised Specification (table 3). ^{13}C-NMR spectroscopy has confirmed (Anderson *et al.* 1991) that, apart from sample F1 (a 50/50 blend of gum arabic with gum talha, for which reference spectra have been published) (Artaud *et al.* 1982, Anderson and Wang Weiping 1990) all were based on *Combretum* alone (figure 5) or *Combretum* admixed with gum talha or gums from other non-permitted botanical sources for which the NMR spectra will be given in a subsequent publication. It is of interest that, of the samples F1–F9 (table 3), samples F1, F2, F7 and F8 would have satisfied the earlier specifications; the attempted supply of such adulterated or misrepresented samples was the underlying reason given for the 1990 Revision (WHO 1990b). Samples F1 and F2 now fail in respect of both the nitrogen and specific rotation values; F7 and F8 fail in respect of the specific rotation value only.

There is no doubt, however, that an unscrupulous vendor could, through more

skilful selection from the commercially available *Combretum* and other non-permitted gums, devise blends that would satisfy the Revised Specification fraudulently through not containing any gum from *Acacia senegal*. There is great financial advantage to be gained; gums are available for such purposes at around only 500–600 dollars per tonne, in contrast to gum arabic from *Acacia senegal* at 2300 dollars. The Revised Specification could easily be made much more specific for gum arabic by slight expansion of the present 'Identification test B' to specify that galacturonic acid, mannose and xylose *must be absent* as well as arabinose, galactose, rhamnose and glucuronic acid *being present*, with galactose the major sugar and rhamnose present in significant, not trace amounts.

The ranges of values now quoted for the nitrogen content and specific rotation in the Revised Specification unfortunately are such as to leave loopholes for commercially attractive value-added blending operations with gum such as gum talha, available at 750–800 dollars per tonne. Whenever a range is quoted in a specification, trade interests dictate that the minimum standard is met. Calculation readily shows that, by using a typical Sudanese gum talha having 0·15% nitrogen and a specific rotation of plus 40 degrees, the following blends would all satisfy the Revised Specification: 90% of N5, N6 or N7 with 10% gum talha; 92% of N9 with 8% talha; 93% of N2 or N4 with 7% talha; 94% of N3 with 6% talha. This would lead to either substantial gains (90–150 dollars per tonne, a very substantial sum for larger traders) or to comparable price reductions to make the adulterated product appear to be more competitive than authentic gum arabic offered by a more ethical competitor. All gum-producing countries market pure gum arabic, mixtures that meet the specification, and more heterogeneous mixtures that do not meet the specification. The Sudan, with its vast agroforestry ecosystems resulting from massive international gum belt re-stocking aid programmes, supplies 85% of the world's supply of good quality gum arabic; it also sells good quality gum talha separately at much lower prices. Most other producing countries, in which gum is collected from more sparse, mixed natural populations of various types of gum trees, are well known by trade buyers to produce the majority of the much more viable, mixed quality, gum parcels which consequently command much lower prices, and are therefore bought largely for blending or technological purposes. As table 4 shows, all minor gum-producing countries can produce gum arabic to meet the Revised Specification. But a trading problem exists for these countries to tighten the gum collection and marketing practices used in the past; previous regulatory specifications did not enforce changes in the traditional practices, but these are no longer adequate.

The Test Article used in studies sponsored by the International Gums Association for Research Ltd, after its formation for that purpose in 1980, was carefully and responsibly selected after taking into account the possible future consequence that its specification may become adopted. The Test Article was therefore the most appropriate that could be devised to represent bulk, general supplies of commercial gum arabic capable of being supplied to food processors by any international gum trader. The validity of the Test Article, as shown originally by comparisons of its published analytical parameters (Anderson *et al.* 1983) was confirmed more recently when they were shown to be in excellent agreement with the mean values established for 35 Sudanese and Nigerian samples representative of production since the early days of this century (Anderson *et al.* 1990). That confirmation is now extended even more powerfully by the close similarity of its

^{13}C-NMR spectrum (sample R12, table 4, and figure 4) to that of some reference samples (R1–R11, table 4 and figures 3, 4) of known origin selected solely to give a wide spread of production seasons and locations. The intensities of the resonances in the Test Article (R12) spectrum are in particularly good agreement (as might be expected of a bulked, representative sample), with the mean values calculated for all other samples studied here ($n = 35$) as shown in table 7.

^{13}C-NMR spectra for gum arabic from *Acacia senegal* have been published previously (Artaud *et al.* 1982, Defaye and Wong 1986, Anderson and Wang Weiping 1990). There is good agreement between these, but the reduced scale of these reproductions makes the precise chemical shifts (ppm) of the resonances difficult to reconstruct precisely and no details have been given of their relative intensities. The complete spectroscopic details given here (tables 5, 6, 7) reveal the extent of the small fine-structural differences that lead to the small variations in the sugar compositions, etc. of different gum arabic samples long known to exist (Anderson *et al.* 1968, 1990) as a result of seasonal and geographical factors. The greatest variations are now readily identified as involving the glucuronic acid (resonance at 175·0 ppm) and rhamnose (resonance at 16·5 ppm) contents. These variations are considered to be remarkably small for such a complex natural product. The use of NMR spectroscopy for detecting with ease the presence of 5% of gum talha (*Acacia seyal*) in admixture with gum arabic (*Acacia senegal*) was demonstrated nearly ten years ago (Artaud *et al.* 1982): this can be extended to the detection of other *Acacia* gums (Anderson and Wang Weiping 1990) and gum combretum (Anderson *et al.* 1991) now that reference spectra have become available (see figure 5).

At present, ^{13}C-NMR spectroscopy is the most powerful analytical method available for the unambiguous identification of gum arabic and for the detection of its admixture, accidental or deliberate, with gum exudates from other botanical origins. Its use here has demonstrated that the Test Article adopted by INGAR (Anderson *et al.* 1983) was entirely representative and responsibly selected. Although the Revised Specification gives a greatly improved measure of safety assurance that would be regarded as acceptable under ethical trading practices by companies voluntarily accepting the principles of Good Manufacturing Practice, the ranges adopted for certain parameters do permit significant adulterations of gum arabic with non-permitted gums and the possibility that gums, or gum blends, from non-permitted botanical origins may be misrepresented as gum arabic by any company wishing to show a cynical disregard for the long-established food safety principles. To deal specifically with such cases, which were suspected and now demonstrated to have been of fairly frequent occurrence, it is suggested that the use of ^{13}C-NMR spectroscopy, a well-established powerful analytical technique, should be incorporated into future specifications, if only to serve as the ultimate referee method.

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The gum exudate from *Combretum nigricans* gum, the major source of West African 'gum combretum'

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Gum samples from six individual *Combretum nigricans* trees and two additional reference samples have been characterized. ^{13}C Fourier-transform NMR spectra show that all have the same structure and confirm that the variations observed in their analytical parameters reflect only small fine-structural differences. NMR spectra also reveal that eight West African 'gum combretum' samples from reputable commercial sources originated from *Combretum nigricans*. This identification is important because gum combretum, which is not permitted as a food additive, has been exploited as an adulterant and misrepresented as gum arabic, for which not even the 1990 Revised Specification is sufficiently rigorous to detect such commercial deceptions. NMR spectroscopy has also shown that the rhamnose and uronic acid contents of gum combretum are located within internal polysaccharide chains. This explains the well-known difference in emulsification functionality between gum arabic, in which all rhamnose and uronic acid groups are chain-terminal, and gum combretum which is, in addition, markedly hygroscopic and characterized commercially by its tendency to 'block' in transit and storage.

Keywords: NMR spectra, gum combretum, emulsification functionality, *Combretum nigricans*.

Introduction

Previous publications have indicated the cosmopolitan occurrence and the botanical and chemical complexities of the genus *Combretum* Loefl. (Anderson and Morrison 1990), the gums from which have long been recognized (McIlroy 1957) to be inferior in quality and functional performance to *Acacia* gums. Nevertheless, gum combretum, which is readily available at very low prices throughout West Africa but is not a permitted food additive, has become used to an increasing extent (Anderson *et al.* 1986). The resulting products have been unsatisfactory in terms of quality and shelf-life because the emulsification functionality of gum combretum, and its tendency to 'block', differ so widely from gum arabic (*Acacia senegal* (L.) Willd.) for reasons that have not been adequately explained in fundamental terms. The recent acquisition of gum from separate *Combretum nigricans* trees, and the greater availability of ^{13}C-NMR facilities, have led to a re-appraisal of what was formerly a difficult analytical problem because of the complexity/variability of *Combretum* gums. More extensive data and clearer conclusions have resulted; these should make differentiation between gum arabic and negative rotation 'gum combretum' easier to achieve in future for regulatory purposes. This is essential; *Combretum* trees are sources of potent cytotoxic (Singh and Pettit 1989) and other pharmacologically active compounds (Pettit *et al.* 1988) and the gum contains intensely bitter and highly pigmented substances extracted from the bark.

Experimental

Origin of gum samples

The collection of six small samples of gum, each from a separate *Combretum nigricans* tree growing at Sadoré, Niger, was arranged by Dr R. J. van den Beldt, Principal Agroforester at ICRISAT, Niamey (samples 1–6). Two larger gum samples from *Combretum nigricans* Lepr. ex Guill. et Perr. (samples 7 and 8) were provided by Mr Oseni, Department of Forestry, Ibadan. Eight commercial samples of 'gum combretum', originating from Mali (samples (a) and (b)), 'West Africa' (sample (c)), Niger (sample (d)), Chad (sample (e)) and Nigeria (samples (f), (g), (h)) were provided by gum importers either for reference purposes or for investigations related to their identity, functionality and suitability for specific applications. The gum samples 1–8 were clear and pale yellow in colour, although their solutions varied in colour from yellow to reddish-brown. Samples (a)–(h) varied greatly in colour, from pale brown to a dark red or dark brown, and their solutions were coloured accordingly.

Analytical methods

The standard analytical methods for the carbohydrate and amino acid moieties of *Combretum* gum exudates have been described recently (Anderson and Morrison 1990). ^{13}C Fourier-transform NMR spectra for 10% gum solutions in D_2O at room temperature were recorded overnight at 50·320 MHz with a Brüker WP200SY spectrometer. All spectra were recorded under identical instrumental operating conditions to facilitate their direct comparison.

Structural location of rhamnose and uronic acids

Gum combretum was degraded (mild, acid hydrolysis) by heating a 2% (w/w) solution in 0·005 M sulphuric acid for 96 h under reflux on a boiling water bath. The degraded gum solution was filtered to remove traces of proteinaceous sediments, dialysed against running tap-water for 48 h, then freeze-dried. The degraded gum, obtained in low yield (*ca.* 20%), was examined by ^{13}C-NMR spectrometry.

Results

The data obtained for the general and polysaccharide-based parameters for reference *Combretum nigricans* samples 1–8 are shown in table 1, and those for 'gum combretum' samples (a)–(h) are shown in table 2. Data for the amino acid compositions of samples 7, 8 and (a)–(h) are shown in table 3. The ^{13}C-NMR data for samples 1–8 and (a)–(h) are shown respectively in tables 4 and 5. Representative ^{13}C-NMR spectra are shown in figure 1 (for samples 2 and 3) and in figure 2 (for samples (c) and (f)). These spectra show the resonances recorded for the range 10–120 ppm. In addition, all samples showed minor resonances at 173–174 ppm (arising from C-6 carboxyl groups). Figure 3 shows the major differences between a representative (average intensities for all samples examined) ^{13}C-NMR spectrum for *Combretum nigricans* gum and that for gum arabic (*Acacia senegal* (L.) Willd.). Figure 4 compares the ^{13}C-NMR spectrum for (a) acid-degraded gum combretum with that (b) for the original gum. To enable a more direct comparison of the differences in relative intensities of the resonances for different samples shown in tables 4 and 5, the relative intensities recorded by the spectrometer for

Table 1. Analytical data for the gum exudates from *Combretum nigricans* gum samples.

	Combretum nigricans samples								
	1	2	3	4	5	6	7	8	Mean
Solubility, %	98	100	100	100	100	100	80	90	96
Loss on drying, 105°C	12·0	11·0	10·0	9·2	9·2	10·4	13·5	10·8	10·6
Total ash, 550°C	2·7	1·7	2·1	n.d.[a]	1·3	2·0	2·4	2·3	2·1
Nitrogen, %	0·15	0·50	0·39	0·44	0·24	0·33	0·31	0·22	0·32
Acetyl, %	3·5	2·0	2·1	3·0	1·3	3·8	3·6	4·2	2·9
Methoxyl, %	0·22	0·20	0·18	0·15	0·17	0·18	0·20	0·20	0·19
Specific rotation, degrees	−34	−61	−39	−82	−64	−48	−46	−53	−53
Intrinsic viscosity, ml/g	39	33	23	19	23	33	40	35	31
Tannin, %	0·10	0·15	0·11	0·23	0·08	0·09	0·90	0·20	0·23
Equivalent weight	1260	1710	1420	2340	1920	1480	1300	1480	1610
∴ Uronic anhydride	14	10	12	8	9	12	14	12	11
Sugar composition after Hydrolysis, %:									
Galacturonic acid	11	8	10	6	7	10	8	7	8
4-*O*-Methylglucuronic acid	1	1	1	1	1	1	1	1	1
Glucuronic acid	2	1	1	1	1	1	5	4	2
Galactose	31	28	25	26	25	24	29	35	28
Arabinose	46	55	54	61	59	55	49	48	54
Rhamnose	9	7	9	5	7	9	8	5	7

[a] Insufficient sample.
n.d. = not determined.

Table 2. Analytical data for commercial 'gum combretum' samples.

	Commercial 'gum combretum' samples								
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	Mean
Solubility, %	95	90	98	98	70	98	95	98	93
Loss on drying, 105°C	10·2	10·1	10·4	13·5	10·0	9·9	10·0	11·6	10·6
Total ash, 550°C	2·5	2·3	1·5	2·0	2·9	1·7	2·3	2·4	2·2
Nitrogen, %	0·32	0·39	0·57	0·46	0·14	0·80	0·45	0·32	0·43
NCF (table 4)	6·45	6·57	6·75	6·53	6·23	6·89	6·49	6·11	6·50
∴ Protein, %	2·1	2·6	3·8	3·0	0·9	5·5	2·9	2·0	2·8
Acetyl, %	3·4	3·9	3·5	3·1	4·1	4·3	3·5	4·8	3·8
Methoxyl, %	0·7	0·3	0·2	0·2	0·4	0·2	0·2	0·2	0·3
Specific rotation, degrees	−29	−41	−54	−71	−32	−63	−33	−41	−46
Intrinsic viscosity, ml/g	25	44	38	35	81	21	33	35	39
Tannin, %	0·3	0·3	0·3	0·3	1·1	0·2	0·3	0·2	0·3
Equivalent weight	1290	1250	1470	1360	1110	1560	1380	1420	1355
∴ Uronic anhydride	14	14	12	13	16	11	13	12	13
Sugar composition after Hydrolysis									
Galacturonic acid	8	10	10	7	7	9	8	7	8
4-*O*-Methylglucuronic acid	4	2	1	1	3	1	1	1	2
Glucuronic acid	2	2	1	5	6	1	4	4	3
Galactose	32	31	32	30	25	36	33	35	32
Arabinose	46	51	49	49	49	48	43	47	48
Rhamnose	8	4	7	8	10	5	11	6	7

Table 3. The amino acid composition of the proteinaceous components (residues per 1000 residues) of *Combretum nigricans* samples 7 and 8 and commercial 'gum combretum' samples (a)–(h).

	Combretum nigricans samples		Commercial 'gum combretum' samples							
	7	8	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
% Nitrogen	0·32	0·22	0·32	0·39	0·57	0·46	0·14	0·80	0·45	0·32
Alanine	75	92	64	76	67	73	72	60	88	91
Arginine	16	24	18	17	15	16	36	9	27	22
Aspartic acid	140	111	64	127	131	93	274	61	140	114
Cystine	26	5	1	0	0	16	0	1	10	2
Glutamic acid	57	78	52	84	86	67	61	70	83	73
Glycine	79	155	80	104	69	88	118	64	119	176
Histidine	22	22	36	21	19	23	29	14	23	27
Hydroxyproline	68	65	123	25	61	46	19	10	6	0
Isoleucine	22	33	22	26	18	22	24	14	36	32
Leucine	37	50	54	41	26	30	38	19	55	49
Lysine	24	34	31	30	18	22	45	15	9	41
Methionine	7	6	18	10	4	5	8	4	7	8
Phenylalanine	33	29	40	31	23	25	29	17	41	32
Proline	160	70	126	143	206	330	38	513	123	89
Serine	72	77	124	71	54	45	73	49	67	80
Threonine	86	60	61	106	140	25	54	63	56	68
Tyrosine	40	46	39	44	32	36	41	21	52	52
Valine	36	43	45	44	31	38	41	26	58	44
NCF[a]	6·66	6·25	6·45	6·57	6·75	6·53	6·23	6·89	6·49	6·11

[a] %N × NCF = % protein.

Table 4. Resonance (ppm) and relative intensity data for ^{13}C Fourier-transform NMR spectra of *Combretum nigricans* gum samples.

Resonance (ppm) (±0·2)	Relative intensities Samples									
	1	2	3	4	5	6	7	8	Mean	SD
109·2	8·49	13·13	11·66	7·16	7·83	6·11	8·82	7·77	8·87	0·24
108·1	3·44	2·87	3·13	3·28	4·19	3·48	4·60	3·55	3·57	0·56
106·7	6·78	5·51	5·68	9·03	7·18	8·55	5·98	6·82	6·94	1·29
103·2	8·96	10·06	8·87	4·43	6·61	6·12	8·15	6·06	7·41	1·89
101·4	3·90	4·50	2·98	2·69	2·04	3·68	2·51	n.r.	3·18	0·87
86·7	1·24	1·86	2·34	2·79	3·00	2·54	1·77	2·21	2·22	0·58
84·0	n.r.	n.r.	n.r.	13·95	12·40	n.r.	11·91	11·42	12·42	1·09
83·7	14·77	16·46	15·02	13·72	11·70	12·96	13·30	12·16	13·76	1·58
81·1	18·49	20·85	17·80	21·02	19·38	18·51	21·05	20·53	19·70	1·32
80·3	10·28	10·16	10·16	7·42	7·70	8·63	7·55	5·74	8·45	1·65
76·4	17·21	17·02	17·38	16·42	14·69	16·61	16·97	16·01	16·54	0·87
75·7	n.r.	8·04	6·96	9·07	9·39	8·50	10·17	9·19	8·76	1·04
75·0	7·32	4·97	5·39	5·59	5·89	6·71	5·90	4·26	5·75	0·96
73·8	n.r.	9·41	10·47	7·39	6·45	9·60	n.r.	n.r.	8·67	1·67
73·2	13·34	12·12	11·11	6·50	8·48	8·74	9·44	7·70	8·31	3·56
70·2	12·80	8·85	8·71	6·26	8·51	8·91	10·19	8·73	9·12	1·84
68·5	11·94	11·96	9·94	7·77	5·16	6·77	8·27	5·65	8·43	2·63
66·3	3·08	5·49	4·58	3·96	3·27	3·38	3·26	2·40	3·68	0·97
62·8	5·32	4·15	3·76	4·86	3·13	5·08	3·02	1·75	3·88	1·22
61·0	25·00	25·00	25·00	25·00	25·00	25·00	25·00	25·00	25·00	
54·3	< 1	3·71	2·08	2·47	< 1	1·02	< 1	< 1	2·32	1·11
48·5	< 1	2·85	2·08	2·46	< 1	1·08	< 1	< 1	2·12	0·76
35·9	< 1	3·98	2·31	2·61	< 1	1·09	1·39	< 1	2·28	1·14
16·4	1·90	1·93	2·25	1·01	1·65	1·31	1·79	1·30	1·64	0·41
Factor	×0·96	×1·0	×1·0	×1·0	×0·98	×1·0	×1·06	×1·10		

n.r. = not resolved.

Table 5. Resonance (ppm) and relative intensity data for ^{13}C Fourier-transform NMR spectra of commercial 'gum combretum' samples.

Resonance (ppm) (±0·2)	Relative intensities Samples									
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	Mean	SD
109·2	8·53	7·68	8·46	8·56	6·34	9·73	10·13	8·36	8·47	1·17
108·1	4·26	3·73	3·77	5·06	3·97	5·12	4·80	5·02	4·47	0·60
106·7	6·37	6·84	6·93	5·69	7·12	6·87	6·62	4·98	6·43	0·73
103·2	7·89	7·19	6·81	8·44	5·30	9·18	10·04	6·60	7·68	1·52
101·4	3·01	1·52	2·56	n.r.	3·22	2·75	3·58	3·16	2·83	0·67
86·7	1·95	1·77	2·06	1·53	2·19	2·39	2·15	1·99	2·00	0·26
84·0	11·58	10·79	11·01	10·96	11·83	11·36	13·28	11·36	11·52	0·79
83·7	11·89	12·38	13·19	13·10	12·25	14·19	15·80	14·47	13·41	1·33
81·1	8·37	20·16	20·24	18·83	18·88	22·28	23·50	20·42	20·33	1·78
80·3	7·69	6·87	7·29	8·50	6·67	8·13	8·19	8·28	7·70	0·69
76·4	16·29	15·41	16·03	13·20	17·91	16·12	19·01	15·54	16·19	1·73
75·7	9·07	8·97	9·22	9·30	7·84	11·43	11·81	9·86	9·69	1·32
75·0	6·39	5·05	5·23	4·45	5·03	5·45	6·65	4·77	5·38	0·77
73·8	9·47	6·85	8·13	7·71	6·38	12·88	n·r·	9·34	8·68	2·18
73·2	10·58	8·95	9·07	9·44	7·20	10·56	12·31	8·50	9·73	1·61
70·2	10·93	9·28	9·22	9·36	8·78	10·90	9·33	8·82	9·49	0·84
68·5	9·63	7·14	6·62	6·30	5·29	8·79	9·73	5·17	7·33	1·84
66·3	2·97	3·29	4·57	3·07	2·65	2·05	6·77	3·64	3·62	1·47
62·8	4·78	2·18	3·48	2·23	3·48	3·38	4·10	3·85	3·43	0·88
61·0	25·00	25·00	25·00	25·00	25·00	25·00	25·00	25·00	25·00	25·00
54·3	< 1	1·18	2·57	< 1	< 1	6·66	2·41	2·20	3·00	2·11
48·5	< 1	0·84	2·26	1·81	< 1	5·77	1·93	1·52	2·35	1·74
35·9	< 1	1·23	2·93	2·17	< 1	7·70	2·39	2·04	3·08	2·33
16·4	1·63	0·95	1·44	2·04	2·28	1·47	2·43	1·17	1·68	0·52
Factor	×1·07	×0·95	×0·95	×1·07	×1·0	×1·08	×1·26	×0·97		

n.r. = not resolved.

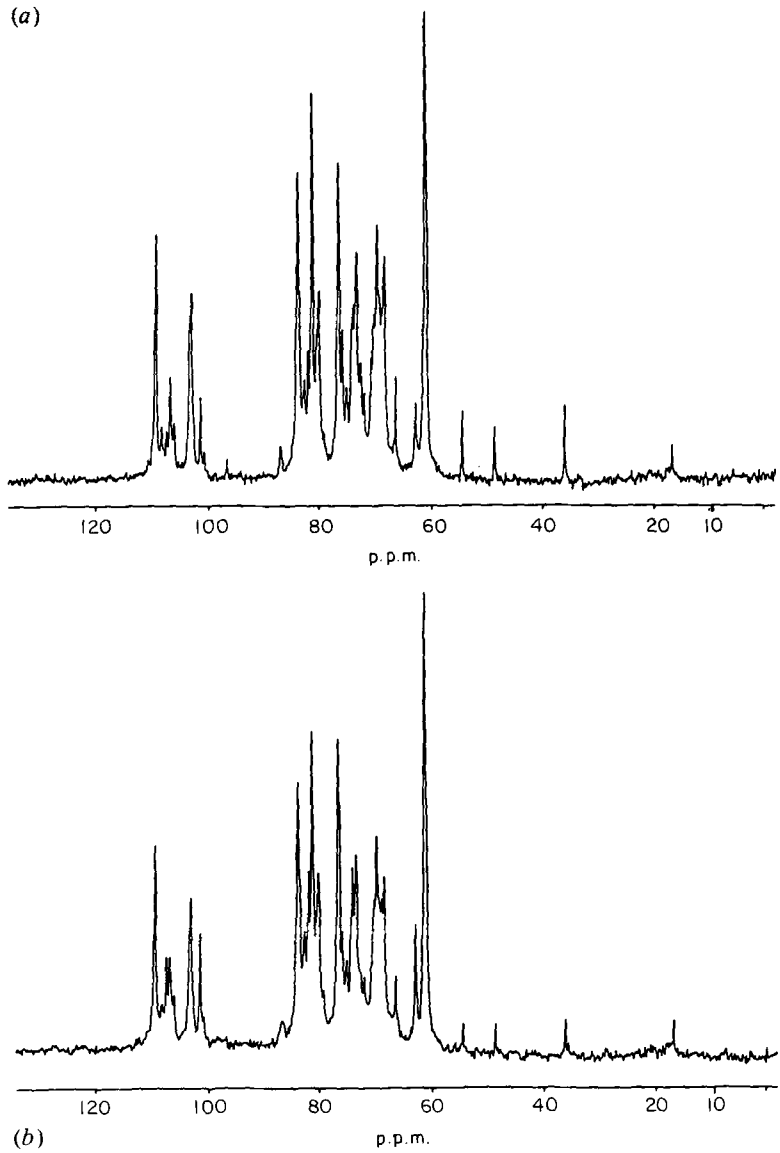

Figure 1. (a) ^{13}C-NMR spectrum for *C. nigricans* sample 2, (b) ^{13}C-NMR spectrum for *C. nigricans* sample 3.

sample solutions, differing slightly in concentration, were multiplied by the conversion factors shown in these tables so as to make the intensity of the principal resonance equal for all samples.

Discussion

The genus *Combretum* Loefl. is the largest and one of the most complex in the Family Combretaceae (Family Myrtales). Taxonomic difficulties have arisen because of its complex, heterogeneous population in which there appears to be a continuous re-shuffling of genes, with a number of morphological characters found

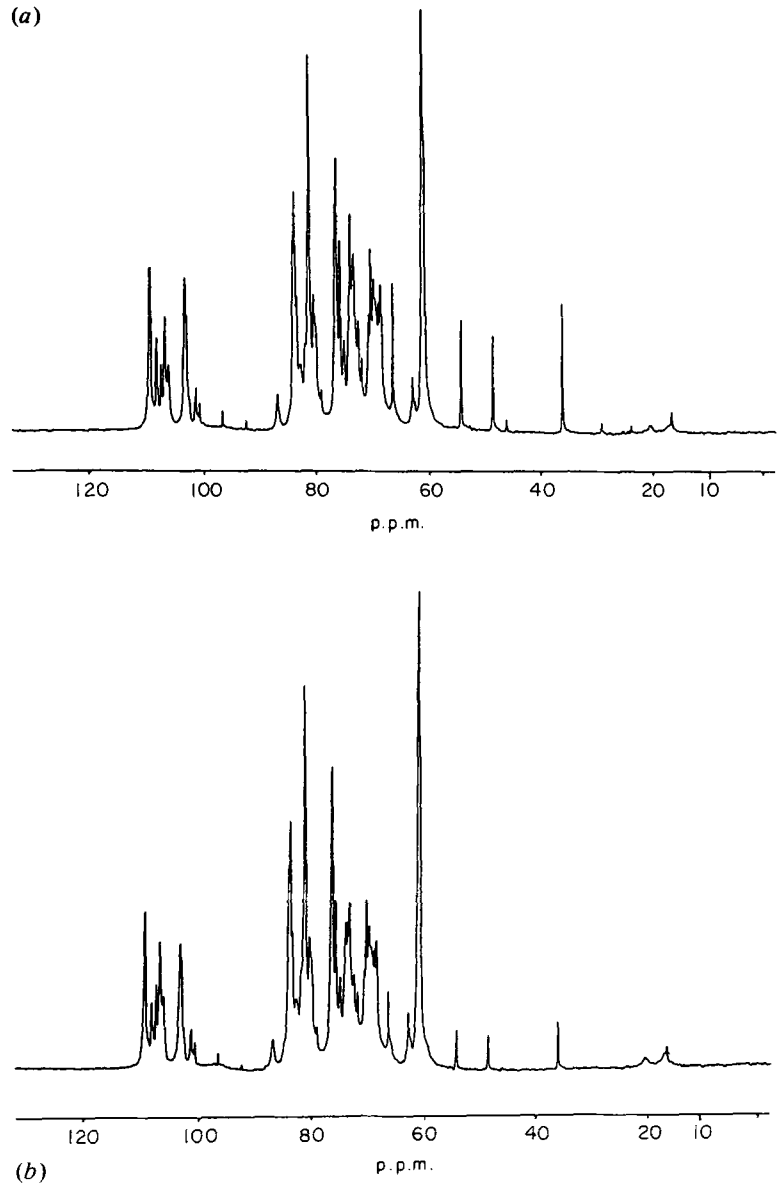

Figure 2. (a) ^{13}C-NMR spectrum for gum combretum sample (b). (b) ^{13}C-NMR spectrum for gum combretum sample (c).

in nearly every possible combination (Exell 1953). As a result, the more complex species can best be regarded as complexes or aggregates. Some aggregates (e.g. *C. collinum* Fresen.) were given as many as fifteen synonyms by different field botanists, with 11 sub-species also being recognized; it has been suggested (Exell 1970) that there is no way of avoiding the inconvenience of changing the preferred name for species to eliminate the extensive use of synonyms. A brief overview of this situation from the gum chemistry standpoint has been given (Anderson and Bell 1977).

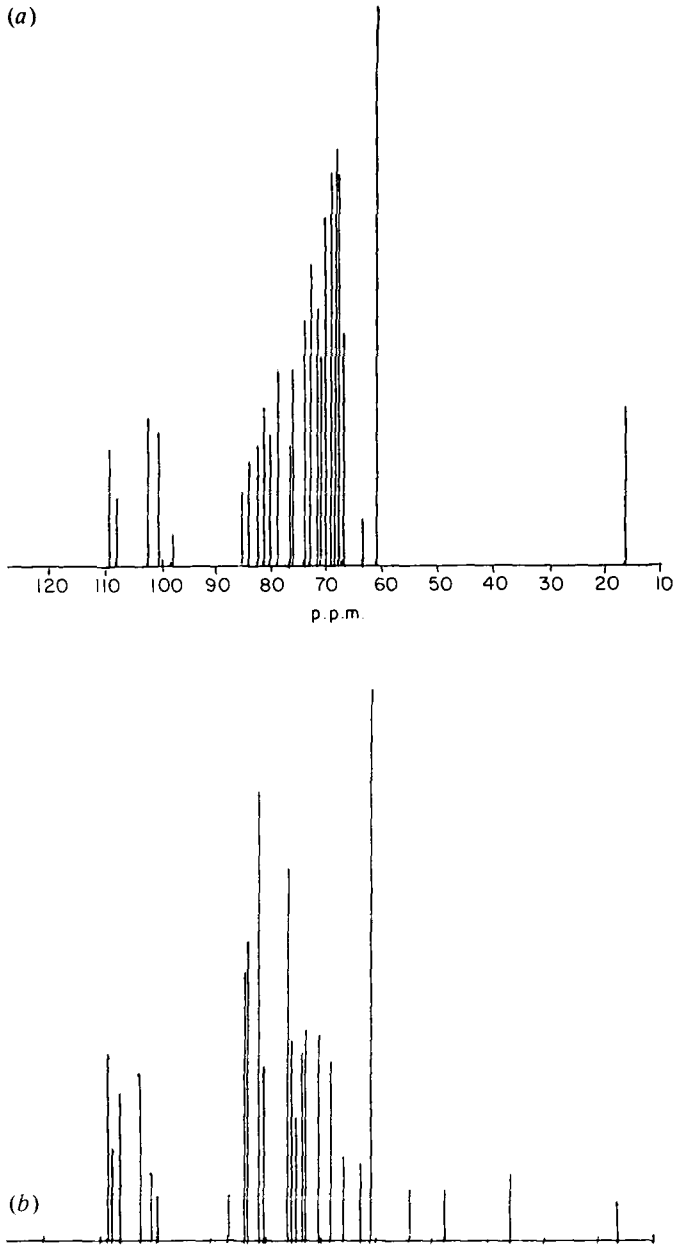

Figure 3. (a) Spectrum representative of gum arabic (*Acacia senegal*). (b) Spectrum representative of *C. nigricans* gum.

For the first time, however, it has been possible to ascertain the extent of the resulting variability in gum chemistry parameters for the *Combretum nigricans* aggregate, in terms of the analytical differences between the six samples collected by hand from authenticated individual trees in Niger, where the *Combretum nigricans* aggregate predominates. The extent of the variability is comparable with that recorded for *Combretum leonense* in the earliest study of this type (Anderson

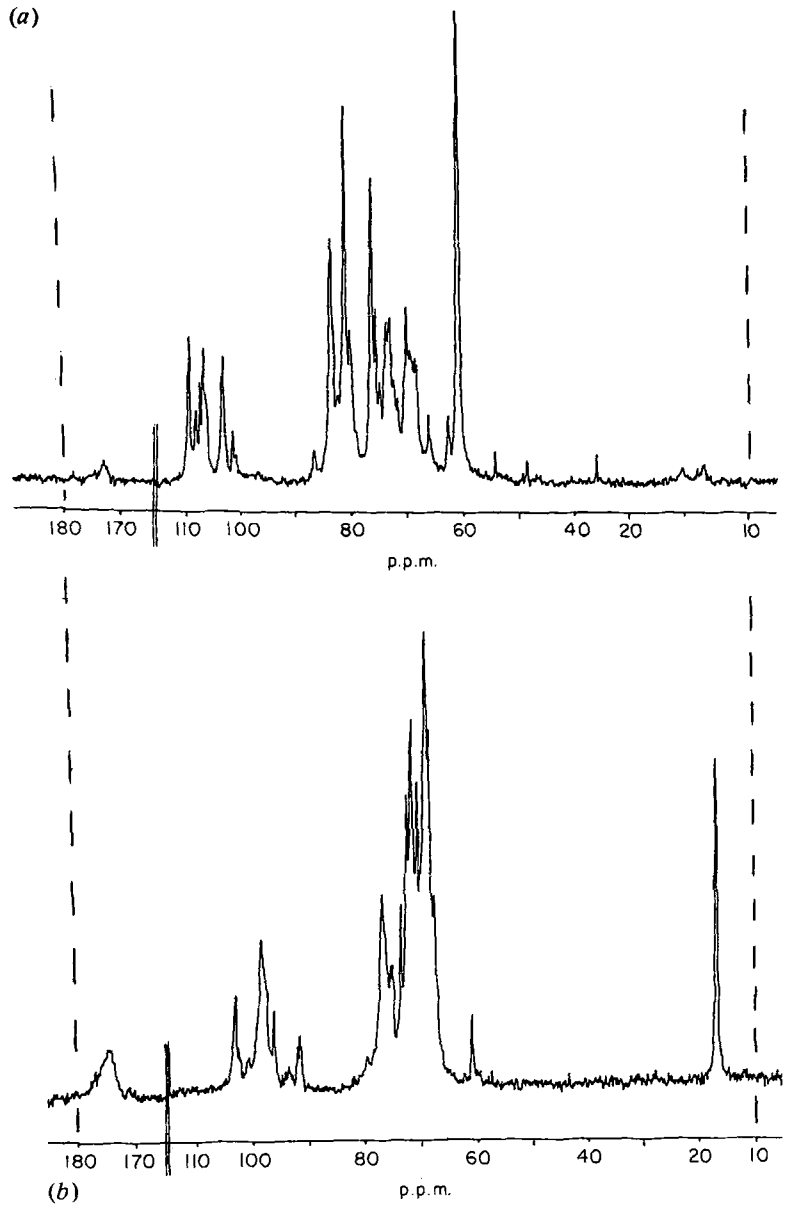

Figure 4. (a) Original *C. nigricans* gum. (b) Acid-degraded *C. nigricans* gum.

et al. 1959). The inter-tree variability for *Combretum* species is much greater than has been encountered in gum samples from *Acacia senegal* (L.) Willd. (Anderson *et al.* 1990) and from other *Acacia* species for which such studies have been made, e.g. *Acacia nilotica* (Anderson and Karamalla 1966) and *Acacia laeta* (Anderson and Smith 1967). Table 1 shows that there are relatively wide variations in the nitrogen content (0·15–0·50%), specific rotation (−34 to −82 degrees), and arabinose/galactose ratio (1·48 to 2·36) of the single tree samples 1–6. As might be expected, samples 7 and 8 (larger, bulk samples comprising gum collected from

many *C. nigricans* trees) correspond much more closely to the mean values. In addition to the neutral sugars reported in table 1, trace amounts of mannose were also detected in samples 1–8 (and in samples (a)–(h), table 2). Table 3 shows a comparable variability in the amino acid compositions and confirms previous indications (Anderson and Morrison 1990) that *Combretum* gums differ from *Acacia senegal* (gum arabic) in having much lower proportions of hydroxyproline and higher proportions of proline, glycine and aspartic acid.

Reference to the corresponding data (tables 2 and 3) for the commercial gum combretum samples (a)–(h) reveals remarkable similarities to the ranges and mean values found for the reference samples 1–8 (table 1). Thus nitrogen contents range from 0·15 to 0·50 (mean 0·32) (table 1) and from 0·14 to 0·80 (mean 0·43) (table 2); specific rotations range from −34 to −82 (mean, −53 degrees) in table 1 and from −29 to −71 (mean, −46 degrees) in table 2. Similar comparisons between tables 1 and 2 can be made in respect of the rhamnose and uronic acid contents, and also for the arabinose/galactose ratios. In terms of these analytical parameters, the major bases for distinguishing gum combretum from gum arabic (Anderson *et al.* 1990) involve the presence in gum combretum of galacturonic acid and traces of mannose, of significantly more arabinose than galactose, and of acetyl and tannin contents, with its intrinsic viscosity being much higher than that of gum arabic. Except for the tannin test, none of these positive bases of differentiation appear in the Revised Specification for gum arabic (FAO 1990), which is intended simply as a means of differentiating between different purities of gum arabic in terms of compliance with the quality of a toxicological test article, rather than as a basis for detecting fraudulent trade misrepresentations of identity which, in terms of the code of Good Manufacturing Practice, are not expected to be attempted. Notwithstanding, details of an additional test for differentiating between gum arabic and *Combretum nigricans* gum have been described (Connolly *et al.* 1988), and even more conclusive methods are outlined below.

Variations in the analytical parameters of different gum samples from one particular botanical species have been reported on numerous occasions (three such studies already cited) in the past. In these, it has always been difficult to ascertain whether such variations represented minor fine-structural, differences or more significantly different polysaccharide structures. This problem has now been resolved through a facility within this laboratory which enables the ^{13}C Fourier-transform NMR spectrum to be obtained overnight for gum samples worthy of fundamental study. Table 4 summarizes the spectroscopic data obtained for reference samples 1–8. The reproducibility of the locations of the recorded resonances (ppm) is remarkable, being within ± 0·1 ppm for several of them. There is therefore a clear indication that the different samples of *Combretum nigricans* gum have a common basic structure and that the variations in their sugar compositions (table 1), as reflected in the variations in the normalized relative intensities of each of the resonances, represent only small fine-structural variations. The recognized structure assignments can be summarized (Anderson and Weiping 1990) as: C-methyl groups in rhamnose (16·4 ppm); —CH_2OH groups at C-6 of hexoses and C-5 of pentoses (61·0 ppm); —CHOH groups at C-2–C-5 (66–86 ppm); anomeric-CH groups at C-1 (101–109 ppm). There is a good correlation between the rhamnose content found by sugar analysis (table 1) and the relative rhamnose contents indicated spectroscopically at 16·4 ppm. The resonances at 35·9, 48·5 and 54·3 ppm correspond to aliphatic —CH, —CH_2, acetate groups,

etc. but may also reflect the presence of some components of natural pigments; it was observed that samples with the higher relative intensities for these resonances were the more strongly coloured. An additional structural insight is available from the resonance at 109·2 ppm and 108·1 ppm, attributable (Defaye and Wong 1986) to internal and terminal arabinose residues respectively: thus the ratio between these is indicated to be 5/2, on average, for the *Combretum nigricans* aggregate gums.

When the commercial samples of gum combretum were examined spectroscopically, the very close correlation between the precise locations of their resonances (ppm) and those of the reference samples for *Combretum nigricans* was not anticipated, although the general similarities of the average values calculated for the sets of parameters in tables 1 and 2 had attracted attention. There is remarkable agreement between the mean values calculated for the relative intensities recorded for all samples in tables 1 and 2; representative spectra are shown in figures 1 and 2 to facilitate visual comparisons. This indicates that the commercial gum combretum samples (a)–(h) are very largely composed of gum originating from *Combretum nigricans*; only samples (a), (b), (c) and (g) gave a few additional spectroscopic resonances indicative of the presence of small amounts of other gums. The strong correlations with *Combretum nigricans* gum is supported by the fact that the ^{13}C-NMR spectra for the gums from other widely occurring *Combretum* species (e.g. *C. paniculatum*, *C. collinum*, *C. fragrans*) differ greatly. Reference spectra for all other species of *Combretum* gum available will be published elsewhere in due course. Meantime, the calculated average relative intensity values in tables 4 and 5 have been used to synthesize the spectral pattern shown in figure 3a, which can therefore be regarded for reference purposes as a representative NMR spectrum for *C. nigricans* gum; the extent of its fundamental differences from gum arabic (*Acacia senegal*) is revealed in figure 3b.

Finally, NMR spectroscopy has been used to establish the nature of the structural differences that account for the marked differences in emulsifying power long known commercially to exist between gum arabic and gum combretum. NMR spectroscopy (Defaye and Wong 1986) confirmed a previously well-established structural feature for gum arabic, obtained from controlled chemical degradations (e.g. Street and Anderson 1983), in which all rhamnose residues occupy chain-terminal positions and are attached to the glucuronic acid groups, i.e. the rhamnose and acidic groups occupy peripheral positions in gum arabic. In contrast, gum combretum is drastically degraded (low yield) in acidic solution; the resulting degraded polysaccharide (figure 4a) contains much higher proportions of rhamnose (16·5 ppm) and of uronic acid (175 ppm) than the original gum (figure 4b). Such enrichments can only have arisen if the rhamnose and uronic acid(s) are located within the core of the original gum molecules and not at their periphery. The presence of 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose has been reported in *Combretum leonense* gum; it was noted that when galacturonic acid occurs in plant gums, it frequently occurs alone or interspersed with rhamnose in the interior chains of the complex molecular structures (Aspinall and Bhavanandan 1965).

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ACACIA SEYAL AND *ACACIA SIEBERANA* – SOURCES OF COMMERCIAL GUM TALHA IN NIGER AND UGANDA

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SUMMARY

Because of the high cost of food grade gum arabic (negative optical rotation, from *Acacia senegal* (L.) Willd.), there is a growing international demand for good quality gum talha (positive optical rotation) for technological applications, available from trees (*Acacia seyal* and *A. sieberana*) growing in Niger and in Uganda (*A. seyal*). There are good opportunities for agroforestry developments in these countries if increased attention is given to meeting the modern marketing requirements of gum trading.

INTRODUCTION

International gum trading patterns are changing. Demand for laevorotatory food grade gum arabic (*Acacia senegal* (L.) Willd.) has decreased steadily from *ca.* 70,000 tonnes p.a. in 1970 to around 25,000 tonnes in recent years whereas production of gum talha, i.e. dextrorotatory gum derived from globose-flowered *Acacia* spp. with prickles in pairs (e.g. *A. seyal, A. sieberana, A. hockii, A. ehrenbergiana, A. karroo*), has remained fairly constant at around 6,000 tonnes p.a. This arises because gum arabic at its present cost (2,300 U.S. dollars/tonne ex Port Sudan) is not cost-competitive against the wide range of modified starches now available; and because the agroforestry developments of *Acacia senegal* concentrated in the Sudan through international aid/redevelopment programmes, which give it a near-monopolistic (*ca.* 85%) share of the world's gum arabic production, do not create market confidence because of the Sudan's vulnerability to drought, famine and religious/

political instability. As a result, there is a marketing desire for production to be more diversified throughout the Sahel; the resulting opportunities for countries such as Niger have been described recently (Anderson et al, 1991). A second opportunity arises, however, in respect of a separate commodity, gum talha, for those countries (e.g. Niger, Uganda) which have natural populations of suitable *Acacia* spp. (e.g. *A. seyal* Del. and *A. sieberana* DC) growing under conditions naturally conducive to production of good quality gum. This report presents data obtained for gum talha samples obtained from *Acacia seyal* and *A. sieberana* trees growing in Niger and *A. seyal* trees in Northern Uganda.

MATERIALS AND ANALYTICAL METHODS

Origins of gum samples

Gum samples from four *Acacia seyal* Del. trees (Samples A, B, C, D) and two *A. sieberana* D.C. trees (Samples E, F) were collected in December 1990 at Sadoré, Republic of Niger, by Dr R.J. van den Beldt, ICRISAT, Niamey. Three samples of gum from *A. seyal* Del. trees (Samples G, H, J) growing at Longiro, Karamoja Region, Northern Uganda, were collected in April 1991 by District Forest Officers.

Analytical methods

The standard analytical methods for characterizing gum exudates have been described (Anderson and Morrison, 1990). Fourier-transform ^{13}C-N.M.R. spectra for 10% gum solutions in D_2O at room temperature were recorded overnight at 50.320 MHz with a Brüker WP200SY spectrometer. All spectra were recorded under identical operating conditions to facilitate their direct comparison.

RESULTS

Table 1 shows the analytical data for the gum samples from *Acacia seyal* growing in Niger and Uganda; data for a Sudanese sample (Anderson et al, 1984) are included for comparative purposes. Table 2 shows the corresponding data for the two samples from *A. sieberana* growing at Sadoré, Niger; published data for *A. sieberana* gum

TABLE 1
Analytical data for gum samples from *Acacia seyal* trees in Niger and Uganda.

	Niger Samples				Ugandan Samples			Sudan*
	(A)	(B)	(C)	(D)	(G)	(H)	(J)	Sample
Loss on drying, 105°C (%)	11.9	11.9	11.7	12.9	14.4	16.3	15.9	13.4
Total ash (550°C) (%)	2.4	2.7	2.2	3.3	3.0	1.6	3.9	2.8
Nitrogen (%)	0.24	0.16	0.13	0.22	0.10	0.11	0.25	0.14
Methoxyl (%)	0.15	0.18	0.17	0.20	0.27	0.30	0.31	0.94
Specific rotation (degrees)	+58	+58	+62	+54	+58	+69	+59	+51
Intrinsic viscosity (ml/g)	21	15	14	19	17	17	16.5	12
Equivalent weight	1200	1260	1690	1525	1230	1275	1550	1470
Hence uronic anhydride (%)	15	14	10	11	14	14	11	12
Sugar composition after hydrolysis								
4-*O*-methylglucuronic acid	1	1	1	1	2	2	2	5
Glucuronic acid	14	13	9	10	12	12	9	7
Galactose	34	37	37	36	34	34	37	38
Arabinose	49	47	52	51	48	50	49	46
Rhamnose	2	2	<1	2	4	2	2	4

* Data from Anderson et al (1984)

TABLE 2
Analytical data for gum samples from *Acacia sieberana* trees in Niger

	Niger Samples (E)	Niger Samples (F)	Sudan* Sample	Ghana* Sample
Loss on drying, 105°C (%)	10.8	10.3	7.1	6.0
Total ash, 550°C (%)	1.0	0.7	1.9	1.5
Nitrogen (%)	0.27	0.40	0.35	0.19
Methoxyl (%)	0.16	0.14	0.74	0.68
Specific rotation (degrees)	+106	+114	+106	+103
Intrinsic viscosity (ml/g)	11	13	12	12
Equivalent weight	1825	2200	2300	1230
Hence uronic anhydride (%)	10	8	8	14
Sugar composition after hydrolysis				
4-*O*-methylglucuronic acid	1	1	4	4
Glucuronic acid	9	7	4	10
Galactose	33	32	28	35
Arabinose	57	60	60	47
Rhamnose	<1	<1	4	4

* Data from Anderson et al (1984).

originating from the Sudan and from Ghana are also shown. Table 3 presents the amino acid compositions and Table 4 gives the N.M.R. data for the gum samples (*A. seyal* and *A. sieberana*) obtained from Niger. Figure 1 shows the full spectra for *A. seyal* samples (B) and (D); Figure 2 permits a visual comparison of the spectrum for *A. sieberana* gum sample (E) with that for the sample of gum arabic (*A. senegal*) used as the toxicological Test Article (Anderson et al, 1983).

DISCUSSION

All of the samples studied were of excellent quality, being completely soluble in cold water, giving solutions of good viscosity, clarity and colour. All confirm the previously well-established (Anderson, 1978) analytical characteristics of gum talha derived from such *Acacia* spp. (Series *Gummiferae*) viz. highly positive optical rotations, higher proportions of arabinose than of galactose, and low rhamnose contents. These characteristics are the opposite of those shown by

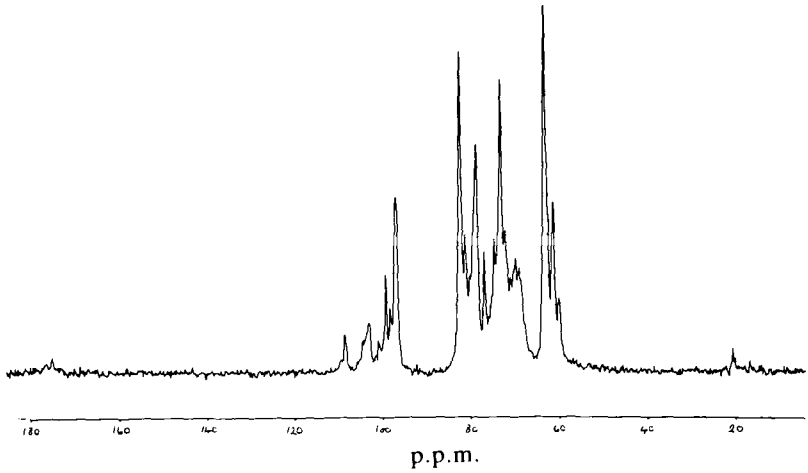
TABLE 3
Amino acid composition of *Acacia seyal* and *Acacia sieberana* gum samples from Niger

	Acacia seyal				*Acacia sieberana*	
	(A)	(B)	(C)	(D)	(E)	(F)
Nitrogen (%)	0.24	0.16	0.13	0.22	0.27	0.40
Alanine	32	40	42	32	42	41
Arginine	15	19	20	14	16	59
Aspartic acid	44	49	65	41	75	71
Cystine	4	0	0	16	12	0
Glutamic acid	39	55	73	35	63	63
Glycine	42	46	52	35	47	48
Histidine	48	49	40	44	20	18
Hydroxyproline	277	248	236	289	253	228
Isoleucine	12	13	16	12	26	24
Leucine	65	70	62	65	51	49
Lysine	20	19	23	15	23	56
Methionine	1	2	3	2	3	3
Phenylalanine	27	8	43	27	52	47
Proline	76	82	56	79	75	73
Serine	188	184	154	183	92	78
Threonine	55	61	55	55	63	58
Tyrosine	17	14	17	16	26	24
Valine	38	41	43	40	61	60
Hence Nitrogen Conversion Factor	6.52	6.40	6.59	6.62	6.96	6.24

gum arabic (*Acacia senegal*); this explains the great differences between the functionalities of gums arabic and talha. There are no toxicological studies of the safety of gum talha. It is not included in any of the regulatory permitted food additive lists, and has always been marketed by the Sudanese as an entirely separate commodity at *ca.* 30% of the price of gum arabic.

Nevertheless, there are commercial applications for which gum talha is completely satisfactory; tonnages could be expected to increase if its quality/consistency could be improved. Traditionally, gum talha has been sold as a low-grade gum of very variable identity,

(a)



(b)

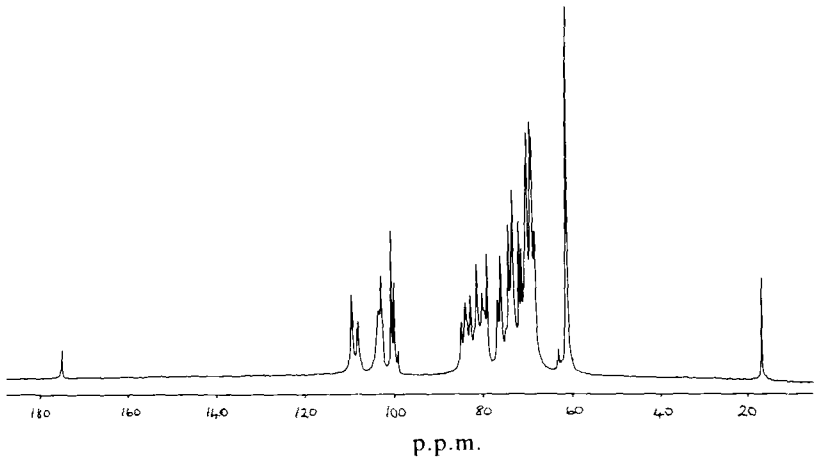


Figure 2. ^{13}C-N.M.R. spectra for: (a) *Acacia sieberana* gum sample E from Niger (b) *Acacia senegal* (gum arabic).

composition and quality. This results from the admixture of small parcels of gum from different geographical locations and botanical sources, not infrequently including sub-standard dark or dirty gum arabic which cannot be sold for food use. Good quality is essential as much in terms of self-consistent, reproducible functionality as in terms of its solubility, pale colour, freedom from sand, etc. Consistent functionality/quality from season to season can best be achieved when the gum originated from a single *Acacia* source. In contrast to gum arabic production, arising from the vast Sudanese monocultures, this is not easy to attain for gum talha which has never been involved in afforestation programmes and therefore can only originate from locations endowed with natural, mixed populations of *Acacia* spp. which may also be interspersed with other gum-yielding genera, e.g. *Albizia*, *Combretum*.

There is therefore great scope for increasing gum production in selected areas where a single *Acacia* species predominates, or where small proportions of other *Acacias* can be eliminated, or where control over the species tapped/collected can be exercised. Opportunities for this now appear to exist in regions of Niger and Uganda, i.e. locations not previously prominent in gum production. It is particularly important for the gum collectors to be made keenly aware of the fact that gum from a single botanical source will meet more ready demand if it is not admixed with gum from other species and if that gum is available in regular supply so that its optimum suitability for specialised technological applications can be established.

Acacia sieberana is seen (Table 2) to be less acidic and less viscous with a higher positive rotation than the gum from *A. seyal* (Table 1), although both these gums are similar in respect of their low rhamnose content and high arabinose/galactose ratios. Table 3 reveals that the amino acid compositions of the *A. seyal* and *A. sieberana* gums are broadly similar although *A. sieberana* has higher proportions of aspartic acid and valine, and lower proportions of histidine and serine. The latter is unusually high in *A. seyal*: this has been shown (Anderson and Yin, 1988) to reflect the location of higher proportions of amino acids at the core of the highly branched gum molecules than at peripheral sites considered to be responsible for surface active functionality (Dickenson et al, 1988) and this is one of its major differences from gum arabic.

A Revised Specification for gum arabic (*Acacia senegal*) was published in 1990 (FAO, 1990); a range of values for its specific

rotation (minus 26° to minus 34°) and for its nitrogen content (0.27 to 0.39%) was proposed with the intention of restricting the feasibility of adulteration with gums from unproven, non-permitted botanical origins, e.g. gum talha. Although the Revised Specification has been challenged by organised trade interests and has not yet been approved by the Codex Alimentarius, the regulatory authorities have confirmed the basic principle of food safety that the identity of the article of commerce must reflect that of the Test Article tested and approved. This may lead in due course to toxicological evidence of safety and a specification becoming established for gum talha, for which neither exists at present.

The most powerful method of specifying the source, identity and purity of a gum exudate is in terms of its ^{13}C-N.M.R. spectrum: the availability of a good reference spectrum for a particular gum exudate also allows its presence in admixture with a different gum to be detected. The opportunity has therefore been taken to obtain full data (Table 4) for the relative intensities and locations (p.p.m.) of the characteristic resonances given by the gum samples from Niger. Figure 1 shows the full spectra for *A. seyal* samples B and D: these reveal the fine-structural differences between these samples in terms of the varying relative intensities of the major resonances at 81.8, 68.5 and 61.1 p.p.m. Sample A shows a similar profile to sample D, and sample C is similar to that shown for sample B. Figure 2 shows the spectrum for *A. sieberana* sample B. Figure 2 shows the spectrum for *A. sieberana* sample E (sample F is closely similar) and that for a representative sample of gum arabic (*A. senegal*); this permits simple comparisons to be made which reveal the great structural differences between these three gums; *A. seyal* gum is much more complex structurally than *A. sieberana* gum, with these two gums more closely similar to each other than to *A. senegal*.

CONCLUSIONS

Gum talha has the reputation of being a low-grade gum, sold for technological applications at about 33% of the cost of gum arabic. This arises, at least in part, because of the lack of an international specification of identity/purity. "Gum talha" is, in effect, simply a generalised trade description for water-soluble gum from any *Acacia* species other than *Acacia senegal*. The variation in composition and functionality shown by different consignments from different

locations is so great as to make its consistent utilisation in any one repetitive commercial process completely unmanageable and therefore the more self-consistent but more expensive gum arabic must be bought. Locations in Africa such as those described in this report (others may also be identifiable) that are able to produce gum talha that is much more self-consistent in composition, quality, and functionality through collection from a single botanical source, and marketed as such, have an opportunity to derive a new or increased income from natural forest regeneration of the type desirable, particularly when gum tappers/collectors are briefed adequately in terms of the necessity for strict avoidance of admixture of gums from different species. In many African locations, established archaic methods of collecting/marketing gums are no longer adequate to meet the requirements of the more sophisticated and more highly regulated modern market.

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RÉSUMÉ

A cause du coût élevé de la gomme soudanaise de qualité alimentaire (rotation optique négative, obtenue d'*Acacia senegal*), la demande internationale va croissant pour la gomme talha de bonne qualité (rotation optique positive) pour des emplois technologiques. Ce produit peut être obtenu de deux autres essences d'*Acacia* qui poussent au Nigérie: *A. seyal* et *A. sieberana*. On trouve *A. seyal* également à l'Ouganda. On présente des données analytiques à propos de ces essences. Il existe de bonnes occasions de faire des développements agroforestiers dans ces pays à condition de prêter plus d'attention à répondre aux besoins du marketing moderne dans le commerce de la gomme.

RESUMEN

A causa del elevado coste de la goma arábica con grado alimenticio (rotación óptica negativa) de la *Acacia senegal*, existe una creciente demanda internacional para obtener goma talha de gran calidad (rotación óptica positiva) para usos tecnológicos. Esta se obtiene de otras dos especies de *Acacia* que se cultivan en Niger.: *Acacia seyal* y *Acacia sieberana*. La *Acacia seyal* también se produce en Uganda. Se presentan los datos analíticos de estas dos especies. Hay excelentes oportunidades para desarrollar una agrosilvicultura en estos países si aumenta la atención para abastecer los requisitos modernos del mercado comercial de goma.